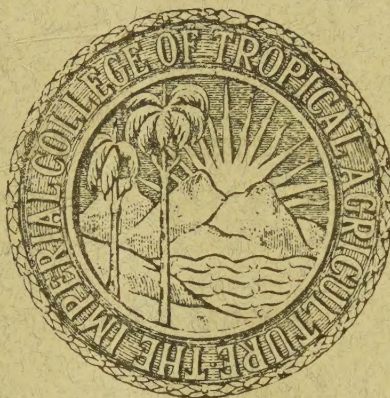
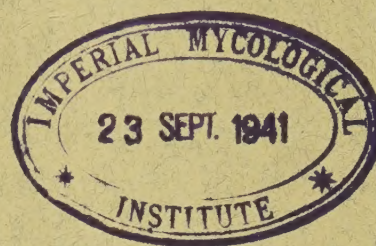


TENTH ANNUAL REPORT ON CACAO RESEARCH 1940



Via colendi haud facilis.

Price

Five Shillings

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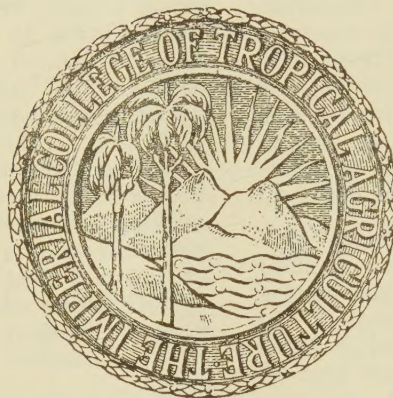
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ANNUAL REPORT ON
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1940-1941

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TENTH ANNUAL REPORT ON CACAO RESEARCH, 1940.

A.—BOTANICAL SECTION.

THE BOTANICAL PROGRAMME OF 1940.

By E. E. CHEESMAN.

Most of our time this year has again been taken up by propagation, planting and maintenance of field experiments, and there is little else of special interest to report. Mr. R. K. McKee, who succeeded Mr. Cope as Botanist for Cacao Research during the year, has started a series of observations on the growth and periodicity of the young clones planted in 1937, but these must run for a full year or more before conclusions are drawn.

For the same young trees (576 planted in the first set of randomized blocks at River Estate) yield records were opened from 1st August, 1940, but owing to the abnormally dry "wet" season, there was no yield worth discussing before the end of the year. The clones, especially I.C.S. 1, set up a very promising show of cherelles in August but lost them in a wave of wilting which had its peak in the latter half of September. Early in 1941 they set heavily again, and promise to mature a good crop so far as numbers of pods is concerned but as they are developing under extremely dry and unfavourable conditions the pods will be small and the yields correspondingly reduced.

The few cuttings which were planted on San Juan Estate in 1936, before we had enough material for the first of the planned experiments, have been kept under observation by the College Department of Economics, and have maintained their highly satisfactory performance. Sixteen 4-year old cuttings of I.C.S. 1 gave for the 1940-41 crop an average yield of 4.5 lb. per tree, and the average yield of 46 young trees, a mixture of four clones some three and some four years old, was 3.3 lb. It may be recalled that some of these trees

photographed at 2½ years old, were illustrated in the 1938 Report. They are in some of the best soil in Trinidad, and we do not expect the same performance everywhere, but as an index of potentialities these figures are impressive.

Having no experimental results to present, we are taking this opportunity to publish details about the field experiments already planted or being planted. The history of their establishment and particulars of their layout will be needed for reference in future reports, and it should prove convenient to have the data collected in one place. The experiments have been numbered serially, and will retain the same numbers as long as they remain in being. By this arrangement, the complete history of any experiment should be traceable through successive annual reports.

In connection with Experiments CRB. 1 to CRB. 6, and some others to be described later acknowledgment and thanks are due to Mr. F. Yates of Rothamsted Experimental Station and Mr. T. N. Hoblyn of East Malling Research Station. In 1936 these gentlemen carefully examined our proposals for field experiments and generously gave us the benefit of their great experience in devising a layout suitable for our problems and conditions. There has been no appropriate occasion until now to make this acknowledgment, and with the passage of time a few details of the layout then agreed upon have been modified in Trinidad, so that for any defects in the experiments as they now stand the writer alone is responsible: for the general plan we remain indebted to the institutions and officers named.

FIELD EXPERIMENTS OF THE BOTANICAL SECTION.

By E. E. CHEESMAN.

1. GENERAL NOTES ON FIELD EXPERIMENTS CRB. 1 TO CRB. 6.

The first six field experiments of the Cacao Research Scheme (Botanical Section) occupy Field 7, River Estate (fig. 1) and as they have all been planted on a similar layout, with similar objects and by similar agricultural methods, they may conveniently be considered together.

Objects.—These experiments were designed to combine comparison of Imperial College Selections with comparison of various methods of vegetative propagation. They are the logical outcome of the selection programme of 1930-35, and constitute the practical test of the methods then adopted.

For any one of the six experiments, the objects may be tabulated as follows:—

(a) To compare quantitatively, with statistical control of results, the performance of six selected clones.

(b) To compare quantitatively the performance of cuttings with the performance of buddings of the same clones on seedling stocks.

(c) To compare quantitatively the performance of trees propagated from fan material (buds or cuttings) with that of trees propagated from chupon material (buds or cuttings).

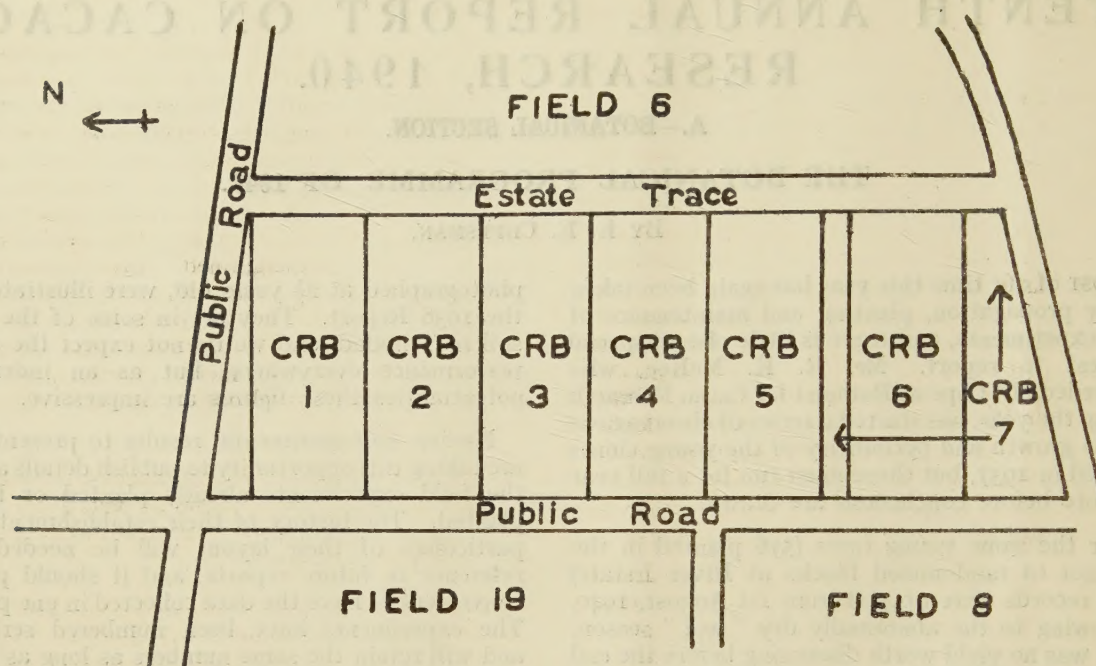


FIG. 1.—Diagrammatic plan of Field 7, River Estate.

(d) To make qualitative observations of the behaviour of the clones concerned, with particular reference to clonal characteristics which may be of agricultural importance.

(e) To obtain in time adequate samples of prepared cacao from each clone for commercial appraisalment.

With regard to object (a), the inclusion of a Standard Clone (I.C.S. 1) in each and every experiment makes possible an indirect comparison between clones in one set and those in another, and should enable us ultimately to arrange all the clones represented in approximate order of merit with reference to their behaviour on this particular site.

Originally it was intended to repeat experiments of this type until the whole 100 selections, or at least a large proportion of them, should have been included. We are, however, suspending this plan with the completion of CRB. 6, for several reasons. The chief of these is that our resources, both in area of land and in propagating capacity, are limited, and there are important new investigations such as rootstock comparisons waiting to be opened.

The six experiments here described include 30 clones and should be sufficient to answer several questions. They should show to what extent the methods adopted in selection have succeeded in isolating productive types; they will indicate the extent to which their own design is adequate for

its purposes; and they provide a sufficient range of different types for the study of interclonal variations in behaviour.

Observations and comparisons of the remaining clones will proceed, but for the time being they will be made on a different plan, which is more fully discussed in connexion with Experiment CRB. 7.

With regard to object (c), it will be noted that comparisons between chupon and fan material occur in only three of the experiments. This departure from the general plan was due to a scarcity of chupon material in the nurseries at the times when CRB. 3, CRB. 4 and CRB. 5 were planted. It was deemed better to get these experiments planted with fan material throughout than to delay them.

Layout.—Each experiment is a set of six randomized blocks, each block of six plots, each plot of 16 trees, making 576 trees in all, or 96 of each clone.

Each plot in a block is planted with a different clone, so that the clone comparison (Object (a)) is based on plot comparison, with six replicates. The plots are square, four trees by four, and each is divided from east to west into two subplots, one containing eight trees propagated as cuttings and the other, eight buddings of the same clone. The position of each type of material north or south of the centre line is determined by chance and comparison of buddings and cuttings (Object (b)) is thus based on subplot comparisons

with 36 replicates. The possible second division into subplots, from north to south, is held in reserve in case it may be needed for answering some new question at a later date.

In those experiments which contain both chupon and fan material, the two classes are segregated into solid blocks. Three blocks, chosen at random are planted entirely with chupon material and the remaining three with fan material. This arrangement gives only three replicates of the comparison which is Object (c), but reduces possible complications due to border effects between trees of rather different habit.

There are no guard rows between plots and blocks, because guard rows add seriously to the area required, and are not considered essential in experiments of this type with tree crops. Guard rows (of I.C.S. 1 cuttings) are, however, planted wherever the outer row of experiment trees would otherwise constitute the edge of the field.

The layout of CRB. 1 is shown in fig. 2, and the layout of a single plot on a larger scale in fig. 3.

Material.—In these experiments the material has always varied slightly in age at the time when it was planted, owing to variations in supply of nursery material, weather conditions affecting propagation, and so on. The cuttings have probably averaged about six months, varying from three to eight months in age. The buddings are older, if we include the time taken to grow the stocks, which is six months at a minimum. The sequence of events which is convenient for good budding does not fit the planting schedule very well, because stocks have to be sown when seeds are available. If stocks are sown in November, and budded about June, they can be put out about October; or if budded later they can be held over till the following June, but it is difficult to have buddings ready in June on stocks which are then only a year old or less.

We have usually followed the first course, and put out the buds rather late, but in CRB. 4 the second procedure was adopted.

For all buddings, the stocks are what we call our "standard mixture". There are ten marked trees on River Estate used for the purpose, and when stocks are required equal numbers of pods are taken from these trees and the seeds are thoroughly mixed before sowing. This procedure prevents the raising of stocks from the few "out of season" pods that might be available, and so limits the flexibility of the budding schedule, but it should have counterbalancing advantages in reducing variation between budded subplots which might arise from the use of different mixtures on different occasions.

Site.—Field 7 is practically flat and level, and was formerly carrying seedling cacao 34 to 40 years old. A sufficient area of this has been felled each year to accommodate the plantings planned for the next year. The average yield of the field for the five years immediately before felling began (1932-36) was 3.4 bags (561 lb.) per acre.

The soil has been sampled by the Chemical Section, and Professor Hardy describes it broadly as follows:—

"The soil of Fields 7 and 6 is fairly uniform in texture and composition, and shows no marked lateral fertility trends. It belongs to the River Fine Sand soil-type, developed from alluvium derived from Northern Range schists and limestone. The soil profile here is red-brown and uniform in colour, becoming slightly paler with depth, and spotted with sepia. The structure is compact and single-grained and somewhat powdery, though very friable and crumbly. Its content of coarse and fine sand is about 50 per cent. the rest being mainly silt with only a small amount of clay. The sand is finely micaceous. Fine schist and quartz gravel occurs sporadically, but stones are rare.

During dry weather, the surface soil tends to dry out and to crack, becoming very hard. In wet weather, water drains freely through it, the water-table being deep-seated.

Texturally, the soil is a fine loamy-sand and uniform to three-or-four-foot depth. It is markedly acidic in reaction (pH 5.6), and definitely deficient in lime. The content of organic matter is medium-low (2.4 per cent.) in the surface 6-inch layer, and falls off rapidly below. The content of nitrogen is medium (0.19 per cent.); the C/N ratio is relatively very low (7.5). The bareness of the soil surface, the paucity of litter, and the low organic status of the soil in these fields suggests serious loss of humic material by sheet erosion during past periods of flood.

The mineral nutrient status is also low, both available phosphate content and available potash content of the surface six-inches of soil being definitely deficient (14 p.p.m. and 55 p.p.m. respectively), diminishing rapidly in the subsoil."

Table 1.

MONTHLY AND ANNUAL RAINFALL IN INCHES AT RIVER ESTATE, 1921-1940.

Monthly.	Average.	Driest.	Wettest.
January ...	3.44	.94	7.31
February ...	1.70	.07	6.36
March ...	2.09	Nil.	6.87
April ...	2.10	.15	7.07
May ...	3.56	.38	11.36
June ...	6.81	3.21	11.96
July ...	9.12	4.13	13.33
August ...	10.04	6.35	15.07
September ...	8.47	3.27	14.06
October ...	7.01	3.74	12.77
November ...	7.82	4.41	14.91
December ...	6.86	1.38	10.63
Annual ...	69.02	57.74	87.15

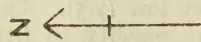


FIG. 3.—Plan of a single plot, to show positions of shade trees and drains. The drains run east and west, and the subplot division (not shown) runs in the same direction down the centre of the middle bed.

Plot 1	2	3	Block 2	Block 3
6	5	4		
Block 6			Block 5	Block 4

[illegible]

FIG. 2.—Layout of Experiment CRB 1 showing, above, the numbering of blocks and plots, and below, the distribution of the six I.C.S. clones and of buddings and cuttings (B = subplot of buddings, C = subplot of cuttings).

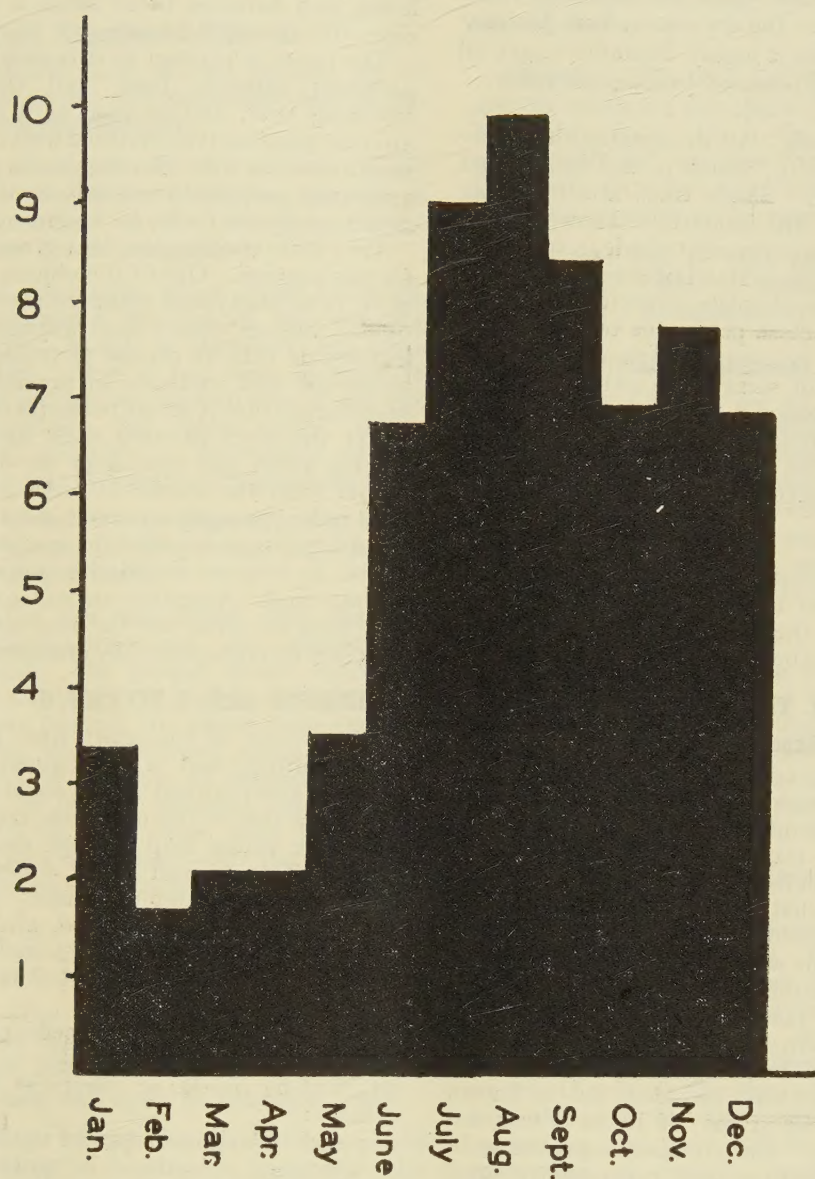


FIG. 4.—Average monthly rainfall at River Estate, 1921-1940.

Some rainfall data for River Estate (1921-1940) are shown in Table 1 and Fig. 4, drawn up from figures kindly supplied by the Trinidad Department of Agriculture. Table 1 illustrates the fluctuations characteristic of the Trinidad climate, and Fig. 4 brings out the feature which has controlled the planting programme—the dry season from January to May, which makes it highly desirable to get all plantings completed between June and October.

Agricultural Details.—All these experiments are planted at 12 × 12 ft., which is the commonest spacing in Trinidad. Shade trees are *Erythrina micropteryx* Poepp., the immortal known locally as "anauca". The permanent shade is spaced at 48 × 48 ft., and the trees stand between cacao rows so that they do not displace experimental trees. This involves very close proximity to the nearest cacao trees, but it is common Trinidad practice, competition does not seem to be serious, and the experiments are designed to eliminate as far as possible any differences between plots in their arrangement relative to the permanent shade. The 16-tree plots have a permanent shade tree at each corner.

For the sake of good establishment of the young crop, the *Erythrina* is planted at 24 × 24 ft., three-quarters of the trees being regarded as temporary shade, to be removed at an early age.

2. THE ESTABLISHMENT OF EXPERIMENTS CRB. 1 TO CRB. 8.

Field Experiment CRB. 1.

Material.—I.C.S. 1, 3, 4, 5, 6, 8, of which 1, 6 and 8 are self-compatible and 3, 4, 5 self-incompatible. The intention at planting was that there should be at least one self-compatible clone beside I.C.S. 1 included in each experiment of this type, in order that the proportion of self-compatibles in the field should be at least one-third. It is hoped that this will eliminate any differential effects of incompatibility on yield such as might be caused by an inadequate supply of suitable pollen, and thus remove one of the factors which might conceivably help to mask true yielding power. I.C.S. 6 was included as a known compatible, but the status of I.C.S. 8 was not known at the time. Since it has also proved to be self-compatible, we have very conveniently equal numbers of compatible and incompatible clones.

Each clone in this set is propagated in four ways, (i) Fan cuttings, (ii) Fan buds, (iii) Chupon cuttings, (iv) Chupon buds.

Planting.—The old cacao on this site was felled in March 1937, and in order to get the experimental plantings started that year the planned procedure was shortened and the first cacao trees were planted on 30th September, 1937, when most of the cuttings were put out. Most of the buddings were planted in December. A few plots were delayed by shortage of material until May, 1938, when planting was completed.

The general practice followed in establishment is that the old cacao and immortelles are felled about May and chopped up on the site, not burned. The land is lined at 12 × 12 ft. and holes are dug to mark the cacao sites. Bananas are then planted at 12 × 12 ft. midway between the cacao holes, and between these again a mixed ground cover of cassava and tania.

The cacao is planted as it is available from the nurseries between June and October of the following year, and as soon as it is well settled after the planting it is mulched with a basket of pen manure to each tree. Planting has in practice varied somewhat each year from this ideal schedule and details are given for each experiment separately.

Very little training has been given to these trees for two reasons. One of the objects of the experiment is to study and compare the natural habits of the various clones and propagation methods, and beside this we do not at present know much about the best methods of pruning this type of material, so that it seems better (at least for a time) to let the trees develop their natural form. A careful watch has been kept for development of shoots from the stocks of budded plants, which need to be promptly removed, and it has also been necessary, since we wish to compare the branch types, to remove occasional chupons arising on fan material. A certain amount of trimming has also been involved by the necessary removal of Witches' Brooms caused by *Marasmius pernicius*.

On account of the short time between felling and planting, and a consequent doubt as to whether good ground shade could be established in time, a few of the old shade trees were left to assist the young crop through the following dry season. This proved to be a mistake, and has been avoided in later plantings. The last of the old immortelles was removed, after poisoning, in July, 1939, but not without some damage to the young trees, which is reflected in the record of "supplies" used.

The number of supplies needed to replace trees damaged by falling immortelles or bananas, badly attacked by beetle, or otherwise spoiled, was as follows:—

1938—	Positions supplied	16 (2.8 per cent.)
1939—	do.	33 (5.7 per cent.)
1940—	do.	4

The percentage, even after our mistake with the immortelles, is gratifyingly low and testifies to the general hardness of vegetatively propagated cacao.

Manuring.—The trees have so far received as manures only pen manure and potash, the latter being regarded as especially necessary on this site.

The pen manure was applied, following the estate practice, as "a basket per tree". This is equivalent to about five tons per acre, and this dressing was applied annually in 1938, 1939 and 1940. Potash was applied as sulphate of potash, 1 lb. per tree in July, 1939 and 1 lb. per tree in February, 1940.

First Fruiting.—A few pods matured in the season 1939-1940, but the crop was too small to warrant individual recording of the trees. Yield records were formally opened on 1st August, 1940, when the trees were almost three years old, and figures for 1940-41 will be available in the next report.

Field Experiment CRB. 2.

Material.—I.C.S. 1, 7, 16, 22, 45 and 53, of which 1, 7 and 45 are self-compatible and 16, 22, 53 probably self-incompatible.

The choice of material for this set was chiefly determined by availability of chupon material in the nurseries, subject to the rule that at least two clones must be self-compatible.

There is an interesting point about I.C.S. 45, which was originally selected in the ordinary Trinitario series, though it obviously has some Criollo affinities. After it had been included in CRB. 2, hand pollinations showed that it was not only self-compatible, but, when selfed, white-seeded. It is probably a descendant of the Nicaraguan trees introduced to Trinidad by Hart.

Each clone in this set is propagated in four ways, as (i) Fan cuttings, (ii) Fan buds, (iii) Chupon cuttings, (iv) Chupon buds.

Planting.—The site was felled with that for CRB. 1 in March, 1937 and not planted till November, 1938. The ground shade, therefore, was good and although the planting was later than could have been desired, the plants came through the dry season, which followed almost at once, quite well. A few subplots of chupon buddings were delayed by lack of material until the dry season had set in, and these were planted in June, 1939 as soon as possible after the first rains. They were soon not noticeably behind the earlier plantings.

The number of "supplies" needed was, in 1939, 28 (4.9 per cent.); in 1940, five.

Manuring.—The trees had pen manure (one basket per tree or about 5 tons per acre) in 1939 and 1940, and 1 lb. sulphate of potash per tree in July, 1939 and again in February, 1940.

First Fruiting.—Yield records will be opened on this experiment on 1st August, 1941, when the trees will be a little less than three years old from planting.

Field Experiment CRB. 3.

Material.—I.C.S. 1, 2, 9, 12, 60, 98, of which 1 and 9 are self-compatible, 2 and 12 self-incompatible, 60 and 98 not finally classified, though 60 is probably incompatible and 98 probably compatible.

The choice was made to cover several different classes of cacao, after I.C.S. 9 had been put in as a known self-compatible. I.C.S. 2 has a thin-shelled pod with very large beans; I.C.S. 12 is a

particularly vigorous specimen of the non-jorquetting Nicaraguan type; I.C.S. 60 was selected in Tobago as possibly drought resistant; and I.C.S. 98 is a heavy bearer of very dark red pods containing second grade beans.

Chupon material was scarce, and these clones were propagated in only two ways, as fan cuttings and fan buds.

Planting.—The site was felled in July, 1938, and the whole set was planted between August and November, 1939. Up to the end of 1940 it had needed only 21 "supplies" (3.6 per cent.) though there may be a few more losses to be made good after the very dry early months of 1941.

The trees had in 1940 the same dressing as CRB. 1 and CRB. 2, namely a basket of pen manure and 1 lb. sulphate of potash each.

Field Experiment CRB. 4.

Material.—I.C.S. 1, 44, 55, 70, 91, 95 all self-compatible. The choice was influenced by the desirability of pushing forward investigations on known self-compatible clones and getting them bulked up so that the best could be recommended for commercial planting as soon as possible. The chupon *v.* fan comparison was omitted, and the clones were propagated only as fan buds and fan cuttings.

Planting.—The old cacao was felled in August, 1939. A very good set of buddings was ready early and all the budded subplots were planted in July, 1940. The cuttings were not all ready, and only 15 of the 36 subplots could be planted; a further 14 were planted in October, leaving seven not completed at the end of the year.

The rainfall for the last four months of 1940 was actually up to average at River Estate, but distributed in so extraordinary a manner that the effect was of a dry season. Both September and November had the lowest figures recorded for those months in the last twenty years (3.27 and 4.41 inches respectively), whilst October with 12.77 inches was by a good three inches the wettest October in the same period. The distribution did not by any means favour planting operations, and there will be a very considerable proportion of replants needed after the 1941 dry season.

The resulting irregularity in age in the subplots of cuttings is unfortunate, and will inevitably reduce the value of comparisons between buddings and cuttings in the early years, but it will not vitiate the clone comparisons, and we may hope that as the whole set ages the effects will gradually disappear.

Field Experiment CRB. 5.

Material.—I.C.S. 12, 45, 54, 85, 101, 104. All these are white-seeded clones, perhaps of pure Criollo descent, and at least classifiable as Criollos

for practical purposes. As the standard clone I.C.S. 1 is purple-seeded, it was thought better to omit it, and to include instead two clones which already occur in earlier experiments, where they can in turn be compared directly with I.C.S. 1. I.C.S. 45 occurs in CRB. 2, and I.C.S. 12 in CRB. 3.

The experiment is not large enough nor sufficiently isolated to eliminate the effects of cross-pollination from purple-seeded clones in adjoining sets, but it is a solid block of white-seeded trees nearly two acres in extent, and we may hope to harvest a fair proportion of pods containing only white beans. Even if we do not, the comparisons of yield and behaviour will still be valid.

Criollo clones were chosen for this set because there is so little reliable information about the behaviour and yield of white-beaned cacaos under Trinidad conditions. But an interesting detail is that four of the six clones were originally selected by Dr. Pound as Trinitario trees—though, as he noted, evidently with Criollo affinities. The parent trees, surrounded by purple-beaned types, showed purple beans and were selected for high yield on the same basis as any others on the I.C.S. list up to I.C.S. 100. I.C.S. 101 and 104, on the other hand, were selected as Criollos without special attention to their yield, beyond the fact that they were bearing well at the time.

I.C.S. 85 differs from the other five clones in having a red pod, and is possibly descended from a Venezuelan parent. It also has very slightly tinted beans, and is probably not a pure Criollo, but it is near enough to be worth including with this set. *This is not the tree described as I.C.S. 85 in the Fifth Annual Report (1935) p. 14.* That tree was lost, and the present one substituted from a reserve list. This is the *only* alteration which has been made in the I.C.S. list since descriptions were published of the first hundred.

The other five clones are almost certainly of Nicaraguan affinity. All are self-compatible except I.C.S. 12.

The comparison between cuttings and buddings is particularly interesting in this experiment, in view of statements that Criollo clones are much more vigorous on Forastero stocks than on their own roots. Some of these types never produce chupons, and so for uniformity the whole set was planted with fan material.

Planting.—Of the 72 subplots, 59 were planted in October 1940 and 13 remain to be completed. The dry November gave the young plants a hard time, and extensive supplying will undoubtedly be necessary after the dry season of 1941.

Field Experiment CRB. 6.

Material.—I.C.S. 1, 25, 31, 36, 67. All these clones are self-compatible, and as in the case of CRB. 4 the choice of material was influenced by the desire to get information as soon as possible about the selections which have been proved self-compatible.

With older material available in the nurseries, a return is being made to the full plan of CRB. 1, including chupon as well as fan material.

Planting.—CRB. 6 is on the 1941 programme and not yet planted. Ground shade is well established on the site, which was felled in September, 1940, and planting should be possible early in the wet season.

Field Experiment CRB. 7.

Field experiment CRB. 7 differs radically from the first six experiments planted, and is perhaps hardly suitable to be dignified by a similar title. Nevertheless some number is necessary to distinguish the planting, and its objects are not without interest.

Field experiments CRB. 1 to CRB. 6 allow for careful quantitative comparisons of 30 clones including the majority of those known to be self-compatible. To plant the remaining 70 on the same plan would occupy most of our propagating facilities for at least five years, and cover about 28 acres of land. At the present stage this does not seem to be the most profitable use of our resources, which can be turned to new investigations whilst we await results from the earlier plantings, and satisfy ourselves that the layout of CRB. 1 is in fact suitable for its purposes, having regard to all the factors that reduce precision in work on cacao.

At the same time, we do need information about the whole 100 clones, and in particular for the purposes of practical application we need to know how they react to different sets of conditions. It is therefore proposed to plant out representative sets of clones in small numbers, but on a range of soil types and under various climatic conditions. Most of these plantings will probably be made by the Trinidad Department of Agriculture, since the observations closely concern Trinidad, but two or three of them will be retained immediately under our own observation, and the first of them is CRB. 7.

CRB. 7 is therefore nothing more than a simple observation plot to accommodate the 70 clones not already planted on a larger scale. There is just enough land left over from the planting of CRB. 1 to CRB. 6 in Field 7, River Estate to take four trees of each clone. No attempt will be made to scatter individuals, as qualitative observations can be made better on small rows or groups of trees than on scattered individuals.

Thirty-six clones have been planted in 1940, in rows of four trees of each, between CRB. 5 and CRB. 6. An irregular plot at the southern end of Field 7, south of CRB. 6, will take four trees of each of the remaining 34 clones in 1941.

The experiment may be regarded as "replicated" by CRB. 9 and by other plots to be laid down as soon as possible. Clones which show promise on several sites will naturally be chosen first for more detailed study when the planting of quantitative comparisons is resumed later on.

The agricultural treatment of CRB. 7 will be exactly the same as that of the rest of the field and need not be repeated here. All the clones will be on their own roots as fan cuttings.

Field Experiment CRB. 8.

Objects.—(a) To study possible connections between self-incompatibility and yield.

(b) To obtain data on the effective range of cross-pollination.

Material.—Four self-incompatible clones, I.C.S. 4, 5, 20 and 97; one self-compatible clone I.C.S. 1. All propagated on their own roots as fan cuttings.

Layout.—The self-incompatible clones are in solid monoclonal blocks, 12 trees long and 9 wide, forming the four quarters of a rectangle, which thus consists of one solid block 24 trees long and 18 trees wide, *i.e.* of 432 self-incompatible trees. The I.C.S. 1 forms a border, three trees deep, round the block; this takes 288 self-compatible trees, and makes the whole planting 720 pickets.

At the time when this experiment was laid down it was assumed that cross-pollination was probably only effective at short ranges, and that if self-incompatibility ever limits yield through failure of pollination, the trees in the centre of this field should show significantly less crop than those nearer to the self-compatible border. Later knowledge suggests that the field may be too small

to show this effect conclusively. The answer remains to be found, but it may be noted now that even if the experiment does prove to be on too small a scale it will still have some value as an indicator of the extent to which incompatible clones can be planted on a commercial scale.

Site.—The site is Field 20D, River Estate, which lies quite close to Field 7 but to the north-east of it across a public road. Most of the agricultural data given for CRB. 1 to CRB. 6 are applicable to this field also. The seedling cacao growing on it prior to 1937 was grown without shade, and the average yield in the five years 1932-36 was 445 lb. per acre.

Planting.—Planting began in August, 1938 and was nearly finished by the end of the year, but not finally completed until November, 1939. Slight age differences between the trees can hardly affect this experiment, and it was planted as material was available, priority being given to the other plantings on hand. During 1939 the field needed 43 "supplies" (6 per cent.) but in 1940 only two pickets had to be replanted.

Records.—Yield records will not be opened for this experiment until all the clones are bearing freely, which may be expected about 1943. The total yield of the field will be taken by the estate authorities, but individual tree records will have little interest until the crop is sufficiently advanced for the effects of pollen supply (if any) to show up.

B.—CHEMICAL AND ECOLOGICAL SECTION.

THE CHEMICAL PROGRAMME OF 1940.

By

F. HARDY.

Staff.

THE biochemical work on cacao was continued during the year by Dr. H. F. Birch, and the ecological and physiological work by Dr. E. C. Humphries. Their accomplishment is indicated by the article and the progress reports that follow. Soil work has mainly consisted of studies on the soil-profile relationships of the root-systems of cacao trees growing in heavy clays, and of soil-aeration, which have been undertaken by members of the staff and Post-Graduate students of the Soils Section of the College Chemistry Department. Progress in this work has not gone far enough to warrant a special report at this juncture.

Manurial Experiments.

No report on the 1940 manurial experiments on cacao in Trinidad and Tobago has so far been issued by the Department of Agriculture, so that further progress in Dr. F. J. Pound's investigations

cannot be summarised this year. It is understood, however, that no fresh experiments have been laid down, that certain old ones have been discontinued, and that plans for a new series of comprehensive experiments, both manurial and cultural, are being contemplated for future application to the main soil-types of Trinidad and Tobago. These experiments, if they materialise, are to be planted up with clonal material, propagated vegetatively from high-yielding strains of cacao.

Physiological and Ecological Investigations.

The six progress reports by Dr. E. C. Humphries that form Part I of this Annual Report describe investigations that follow up those that were described in last year's Report, chiefly concerning the causes of cherelle wilt of cacao.

(1) The observations on cherelle wilt that were carried out at River Estate during 1939 have been continued with some simplifications during another

year. The first results indicated that wilting was mainly caused by competition between developing pods for water and nutrients. Wilting of cherelles was found to be much more pronounced when there were many well-established pods already developing on the tree, but it diminished after the early crop had been removed on ripening. Furthermore, the size of wilted cherelles was found to become progressively smaller as the season advanced until the crop ripened, after which it increased again.

It should be noted that the weather during 1940 was again somewhat "abnormal," in that it differed from that of an average year in having a relatively rainless dry season and a not very rainy wet season. The wet-season months, June, September and November, were again unusually dry, especially September, and these facts should perhaps be taken into consideration in the final interpretation of results. It would appear still to be necessary to continue the observations, until they have at least spanned one average or "normal" year of wetter weather than that which has so far been experienced since this experiment was begun. Nevertheless, it may here be remarked that the direct relationship between fluctuating environmental conditions (such as soil moisture and evaporating ability of the air) and the response-behaviour of cherelles seems not to be marked; the bulky tree might perhaps be considered to interpose a "buffer" between the environment and the developing pods, thus introducing a time-lag, as well as "smoothing out" the reactions of the tree.

In order to obtain additional corroborative evidence regarding the relationship between cropping sequence and wilt, another experiment similar to that at River Estate, was begun at La Reconnaissance Estate, Lopinot, in a field of old cacao growing in the same type of fine sandy soil as that occurring at River Estate, but under a wetter climate. So far, the results obtained in this second experiment support those given by the first.

(2) An additional experiment has been designed further to test the idea that cherelle wilt may be caused by pod competition. The young pods on

two trees were removed, and subsequent setting was compared with that occurring on two other trees that had been left untouched.

(3) Further consideration of the mineral intake by cacao pods during growth (*see* last year's Report) has shown that the greatest intake of minerals occurs when the early crop is maturing, and that this coincides with the period during which the pods that wilt have smallest size, thus signifying great competition for nutrients.

(4) A study of the factors causing variation in the average weight of wet cacao beans per pod at different times of the year led to the conclusion that such variation may be partly attributed to the moisture conditions of the environment as assessed by daily rainfall data.

(5) The metabolic changes accompanying the development of cacao pods are being investigated in an attempt to explain specifically why cherelles of age up to 70 days are more susceptible to wilt than cherelles that have passed that age.

(6) In continuation of investigations on leaf-flushing, a scheme for recording its frequency has been elaborated and applied to a number of selected cacao trees, with the ultimate aim of identifying the responsible factors.

This summary of the objects and main findings of Dr. Humphries' researches is greatly extended in his progress reports, and it is intended to publish the full details of the work in a series of scientific papers when the various investigations have been completed.

Cacao Biochemistry.

The work in biochemistry has concerned the nitrogen transformations that occur in Forastero cacao beans undergoing fermentation. A rapid breakdown of the protein component of the bean was found to occur soon after the cotyledons had been killed by the rise of temperature induced after two days' fermentation, the nitrogenous products being subsequently destroyed and lost, presumably by oxidation or deamination. Full details are given in the article describing this work comprising Part II of this Annual Report.

PROGRESS REPORT ON "STUDIES IN THE PHYSIOLOGY OF THEOBROMA CACAO, WITH SPECIAL REFERENCE TO CHERELLE WILT."

PROGRESS REPORT.

BY E. C. HUMPHRIES.

(1) Repetition of Last Year's Experiments.

In a preliminary experiment (Humphries' *Ninth Annual Report on Cacao Research*) it was suggested that the wilting of young fruit on the cacao tree was primarily due to competition for water and nutrients between the developing fruits, and that fluctuations of the environmental conditions at the particular site (River Estate) had relatively little effect. It was considered desirable

to try to confirm this conclusion with certain modifications at the same site during another season and at a different site having as far as possible a similar soil to that of the first site but situated in a region having a somewhat higher rainfall. Accordingly, a number of trees was chosen for observation at La Reconnaissance Estate, situated in the Lopinot Valley, 11 miles east of Port-of-Spain, where cacao is grown on a

sandy alluvial flat. This soil is essentially the same as that at River Estate where the first experiments were carried out. A block of 100 trees, about 50 years old, was chosen for experiment. Of these, 7 trees were selected for detailed examination while others were examined more superficially at each of the weekly visits.

A modification of the method used in the previous experiment was made in the present case. Each cherelle which had set since the previous visit was tagged with a number and recorded. Because of the labour involved, measurement of the length of pod, the diameter of pod, the distance of pod from base of main trunk and the diameter of the branch at the point where the pod is attached was omitted. The lengths of surviving

Pods were not measured but the condition of each pod on the tree at each visit was carefully noted. If a pod had become wilted or diseased its length was measured and it was removed from the tree.

In addition to the 7 trees at Lopinot, similar records were made on 4 of 14 clonal trees used previously at River Estate, viz.: Nos. 33, 322, 894, 895.

EXPERIMENTAL RESULTS.

Newly set cherelles were tagged in each plot in the second week in July onwards when active setting was just commencing. The number of pods setting in successive weeks followed an irregular course in both plots as was found in the case of the River Estate plot the previous year.

Table 1.

THE AVERAGE SIZE AT WILTING (IN MM.) OF CHERELLES SET IN SUCCESSIVE WEEKS ON 4 TREES AT RIVER ESTATE; SEASON 1940-41.

Date of Setting : 1940-41. Week Ending :—					TREE NUMBERS.				Average All Trees.
					33	322	894	895	
July	16	...	...	...	66	76	57	70	69
	23	...	...	...	58	62	48	69	56
	30	...	...	...	37	45	41	46	46
August	6	...	...	...	37	43	50	37	40
	13	...	...	...	37	40	39	32	37
	20	...	...	...	30	36	33	23	32
	27	...	...	...	32	32	31	22	29
September	3	...	...	...	33	26	31	33	31
	10	...	...	...	33	23	25	30	29
	17	...	...	...	27	24	24	28	26
	24	...	...	...	18	19	19	28	22
October	1	...	...	...	24	20	18	(18)	21
	8	...	...	...	30	11	22	(28)	22
	15	...	...	...	24	(19)	—	(28)	19
	22	...	...	...	(24)	(23)	—	(30)	26
	29	...	...	...	(10)	—	(34)	27	26
November	5	...	...	...	24	—	(11)	(44)	25
	12	...	...	...	28	—	—	(26)	27
	19	...	...	...	(49)	—	—	(102)	79
	26	...	...	...	(51)	(52)	—	—	51
December	3	...	...	...	(54)	—	(25)	90	48
	10	...	...	...	45	(88)	62	—	58
	17	...	...	...	(39)	—	50	(37)	45
	24	...	...	...	(48)	—	54	(65)	55
	31	...	...	...	41	(39)	36	46	40
January	7	...	...	...	35	34	36	33	35
	14	...	...	...	28	(38)	30	(29)	30
	21	...	...	...	29	(14)	22	(23)	22
	28	...	...	...	(22)	—	22	—	22

Figures in parentheses indicate that the average is calculated on three or fewer observations.

In both plots the maximum weekly setting occurred in the first half of August. A secondary maximum occurred in both plots in the first half of September.

The data for wilting have been analysed in a similar manner to that employed in the previous investigation, *i.e.* the average size at wilting of cherelles set in any particular week has been calculated. The results obtained for River Estate agreed very closely with those obtained at this site the previous year. The size at which cherelles wilted became smaller as the season progressed up to the time when the crop on the tree matured, when the size at wilting again became larger. This is brought out in Table 1, which gives the average figures for the four trees under observation. The dates refer to the week in which the pods were set, not to the week in which they wilted. At the beginning of the period (*i.e.* cherelles which were set about 16th July), the average length of cherelles at wilting was 6.9 cm., but cherelles set during October wilted when just over 2 cm. long. The number of young cherelles which not only wilted but also dropped from the tree tended to increase as the season advanced (Table 2). Confirmation was also obtained for the observation made last year, *viz.* :—that, of the pods set in the early part of the season (July), a greater percentage reached maturity than those set subsequently. The progressive decrease in the percentage which became

ripe of the cherelles set in successive weeks is shown in Table 3. After the pods from the first set had ripened the percentage of the pods then setting which subsequently became ripe again increased. Thus the behaviour of the trees at River Estate in the 1940-41 season was almost exactly the same as in the 1939-40 season already reported (Humphries, *loc. cit.*). It should be noted, however, that the figures for the average size at wilting in the 1940-41 season are slightly smaller throughout than those of the 1939-40 season. This is because in the first season the size at which a cherelle wilted was obtained from its growth curve whereas in the second season the size at wilting was recorded as the actual length when examined on the tree when the cherelle had already shrunk an appreciable amount.

In the Lopinot plot much the same results were obtained with an interesting difference. As can be seen from the average* of all trees (Table 4), the size at wilting of cherelles set in the first few weeks decreased in a similar manner to that at River Estate, but after about 6 weeks after the commencement of intensive setting the size at wilting of cherelles then setting again increased and subsequently declined progressively as in the other cases. About August 5th, *i.e.* about a

Table 2.

DROPPING OF CHERELLES AS THE SEASON ADVANCES: RIVER ESTATE, SEASON 1940-41.

Date of Setting : 1940-41. Week Ending :—				Cherelles Shed as per cent. of Total Set.	
July	16	...	...	7	
	23	...	...	13	
	30	...	...	18	
August	6	...	...	18	
	13	...	...	18	
	20	...	...	25	
	27	...	...	27	
September	3	...	...	19	
	10	...	...	23	
	17	...	...	37	
	24	...	...	26	
October	1	...	...	38	
	8	...	...	50	
	15	...	...	45	
	22	...	...	25	
	29	...	...	9	
November	5	...	...	50	
	12	...	...	57	
	19	...	...	62	
	26	...	...	20	
December	3	...	...	25	
	10	...	...	42	

Table 3.

PERCENTAGE OF PODS SET WHICH BECAME RIPE OR DISEASED: RIVER ESTATE: SEASON 1940-41.

Date of Setting : 1940-41. Week Ending :—			Pods becoming Ripe or Diseased, as per cent. of Total Set.	
July	16	...	35	
	23	...	42	
	30	...	2	
August	6	...	1	
	13	...	2	
	20	...	0	
	27	...	0	
September	3	...	0	
	10	...	0	
	17	...	0	
	24	...	0	
October	1	...	0	
	8	...	3	
	15	...	0	
	22	...	0	
November	29	...	27	
	5	...	9	
	12	...	17	
	19	...	20	
December	26	...	0	
	3	...	6	
	10	...	8	

* The average in the last column of Table 4 is the weighted average, *i.e.* the average obtained by taking into account the number of cherelles from each tree.

Table 4.

THE AVERAGE SIZE AT WILTING (IN MM.) OF CHERELLES SET IN SUCCESSIVE WEEKS ON 7 TREES AT LA RECONNAISSANCE ESTATE, LOPINOT; SEASON 1940-41.

Date of Setting : 1940-41. Week Ending :—				TREE NUMBERS.							Average All Trees.
				19	61	55	16	30A	155	42	
July	18 ...	...	...	65	—	76	55	72	73	—	67
	25 ...	...	...	—	(44)	57	36	59	—	(77)	50
August	1 ...	...	...	—	(23)	55	31	43	—	70	51
	8 ...	...	...	25	29	44	26	34	37	73	40
	15 ...	...	...	—	36	35	19	29	61	59	35
	22 ...	...	...	(94)	61	45	21	27	25	69	42
	29 ...	...	...	82	70	38	39	38	—	69	50
September	5 ...	...	...	(60)	—	30	33	37	97	56	40
	12 ...	...	...	42	73	38	24	45	94	44	45
	19 ...	...	...	60	52	36	(22)	63	43	40	45
	26 ...	...	...	(26)	48	34	23	(100)	40	37	38
October	3 ...	...	...	—	52	21	—	42	41	24	37
	10 ...	...	...	—	—	37	31	—	51	49	40
	17 ...	...	...	(38)	(28)	17	36	—	51	34	34
	24 ...	...	...	—	24	28	—	(48)	(42)	—	30
	31 ...	...	...	—	(14)	—	26	(81)	34	—	36
November	7 ...	...	...	—	(14)	19	—	(29)	18	—	19
	14 ...	...	...	—	—	96	—	(22)	(11)	—	64
	21 ...	...	...	—	—	—	(65)	—	(19)	—	(42)
	28 ...	...	...	—	—	(14)	—	—	(25)	—	20
December	5 ...	...	...	—	—	(53)	—	—	—	—	(53)
	12 ...	...	...	—	—	72	—	(11)	—	—	63
	19 ...	...	...	—	—	78	—	—	—	—	78
	23 ...	...	...	—	—	41	—	—	—	—	41
January	2 ...	...	...	—	—	56	—	—	—	—	56

Figures in parentheses indicate that the average is calculated on 3 or fewer observations.

fortnight before the increase in size at wilting of the cherelles began, it was noticed that the leaves of all the trees in the plot were scorched but leaves of trees outside the plot were quite healthy. On June 4th the trees of the experimental plot had been manured with a mixture of potassium sulphate, superphosphate and ammonium sulphate in the ratio of 4 : 3 : 2 (about 4½ lb. per tree). It is suggested that the leaf scorch observed was due to the fertilizer treatment and gives an indication of the time required for it to have an effect on the tree. If this explanation is correct, it also offers an explanation of the rather sudden increase in size at wilting of cherelles set in the latter half of August, a few weeks after the fertilizer scorch was observed. It seems, however, that the fertilizer had no effect on increasing the percentage of pods becoming ripe. This declines throughout the season as at River Estate (Table 5).

There is an alternative explanation of the more irregular behaviour of the trees at Lopinot Estate as compared with River Estate, in regard to size at wilting throughout the season. The trees at

Lopinot represent a mixed population, such as is found in most estates in Trinidad, and consequently there is great variability among individual trees both in time of setting and pod size. For this reason, it is probably inadmissible to average the size of wilting of the separate trees. The figures for the size of wilting for individual trees (*i.e.* the average size of wilting of pods set in a particular week on a particular tree) show a much more regular trend and are then in better accord with the results obtained at River Estate where the work was performed on clonal material. To illustrate this contention, we may take the figures (Table 4) for Tree 55, the tree which set the most pods and therefore gave reliable figures for the average size at wilting in successive weeks. In this case, the progressive downward trend of wilting size is evident, although the fluctuations are greater than was found at River Estate. Similarly in other cases, the figures for individual trees showed a progressive decrease as the season advanced up to the time that pods on the tree became ripe. When the figures for all the trees are averaged, the relationship, in time, of the size

Table 5.
PODS BECOMING RIPE OR DISEASED AND TOTAL PODS SET AT LOPINOT; SEASON 1940-41.

Tree No.	...	19		61		55		16		30A		42		155		Grand Total Pods Set.	Per cent. Ripe + Diseased.
		Ripe + Dis- eased.	Total.	Ripe + Dis- eased.	Total.	Ripe + Dis- eased.	Total.	Ripe + Dis- eased.	Total.	Ripe + Dis- eased.	Total.	Ripe + Dis- eased.	Total.	Ripe + Dis- eased.	Total.		
July	18	...	2	6	7	36	55	15	34	8	16	9	9	6	12	139	60
	25	...	3	3	0	5	25	2	20	0	10	8	9	2	2	71	28
August	1	...	1	1	4	6	57	2	8	0	12	2	4	0	0	90	17
	8	...	9	18	13	7	172	5	47	0	73	8	22	4	16	374	12
	15	...	4	10	6	12	122	0	48	1	87	6	38	7	17	339	11
	22	...	0	4	5	9	86	2	27	0	14	2	15	0	4	156	12
	29	...	0	2	2	2	30	0	6	0	9	2	10	2	3	66	12
September	5	...	0	1	2	0	35	0	6	1	15	0	9	0	3	71	4
	12	...	2	9	0	1	41	0	10	1	12	0	20	0	2	105	4
	19	...	1	3	1	1	25	0	3	1	8	0	11	1	15	87	6
	26	...	0	1	0	4	41	0	5	0	4	0	8	2	36	99	6
October	3	...	0	0	0	0	4	0	1	0	2	0	4	2	19	33	6
	10	...	1	3	0	0	9	0	7	1	2	0	8	0	2	32	6
	17	...	0	3	0	1	5	0	9	0	0	0	3	0	4	26	4
	24	...	0	0	0	1	11	0	1	0	1	0	3	0	1	20	5
	31	...	0	0	0	0	0	0	4	0	2	0	1	0	3	11	0
November	7	...	0	1	0	1	18	0	1	0	1	0	0	2	15	37	8
	14	...	0	0	0	0	4	0	0	0	6	0	0	0	1	12	0
	21	...	0	0	0	1	1	0	1	0	0	0	0	0	1	3	33

at wilting between the trees is such that the curve tends to flatten out and also shows an exaggerated increase at the end of August.

CONCLUSIONS.

The experiments here recorded confirm the conclusion previously reached, viz. :—

- (1) The size at wilting of cherelles set in successive weeks becomes progressively smaller until the time when the crop matures on the tree when it increases again.

- (2) The percentage of pods set in successive weeks which become ripe decreases throughout the season until the crop matures on the tree.

- (3) These results are taken to indicate that cherelle wilt is primarily due to competition between pods for water and nutrients.

ACKNOWLEDGMENT.

I am indebted to Professor Hardy for making detailed observations on the plots during my absence on leave.

(2) The Effect of Removing a Maturing Crop from the Tree on the Subsequent Development of Cherelles.

This experiment was designed with the object of testing the theory that cherelle wilt is primarily due to competition between pods on the same tree.

EXPERIMENTAL RESULTS.

Four trees were chosen for experiment consisting of Nos. 34, 35, 320 and 321 out of the fourteen clonal trees which were kept under detailed observation during the previous year. In the second week in October (which was selected as being a suitable time because pods were then maturing on these trees but had not ripened), all the fruits were removed from Trees 35 and 320. At the same time, all the pods on Trees 34 and 321 were tagged with numbers, and their lengths measured. All four trees were visited subsequently at weekly intervals, new pods were numbered and measured, and the length of each surviving pod measured.

The results obtained for the four trees are set out in the accompanying Table 6. It is clear that the effect of stripping the pods from the trees is that the pods which set in the succeeding few weeks have a much greater chance of reaching maturity than they would have had if the developing crop had been allowed to remain on the trees. The conclusion, however, is not clear cut because the removal of the pods from the trees had an unexpected effect upon setting. As can be seen from the Table 6, the stripped trees set many more pods than the two control trees. Up to December 24th, the stripped trees set 205 (of which 72 or 35 per cent. subsequently became ripe or diseased), while the control trees set only 34 (of which 9 or 26 per cent. subsequently became ripe or diseased). In the subsequent period December 31st to February 26th, the figures are 128 set on the stripped trees and 111 on the control trees—not a very great difference when compared

with the first period. It is suggested that the removal of pods increases setting by one of the three following alternatives :—

- (1) making available more nutrients which causes increased flowering,
- (2) allowing fertilized ovules to develop more readily,
- (3) removing an inhibitor produced by pods already on the tree. Unfortunately no observations were made on the flowering density on any of the trees before or after setting. If, however, explanation (2) is correct it is obviously of importance in connection with tests for self-compatibility.

The contrast between the setting behaviour of the two sets of trees, after stripping the pods from one set, is further emphasised by a consideration of the setting behaviour of these particular trees in the previous season. These figures are shown in the second half of Table 6A. It is evident that here, where there was no interference with the crop, the setting behaviour of the two sets of trees is very similar and, incidentally, very similar to the setting behaviour of the control trees (Nos. 34 and 321), as regards both distribution and total number, in the 1940-41 season.

Owing to the fact that the two control trees remained at a low setting level during the crucial time when it was desired to make a comparison of the treated and untreated trees, the results of the experiment are necessarily inconclusive but strongly suggest that, when developing pods are removed from the tree, the cherelles subsequently setting have a better chance of reaching maturity than if the pods had not been removed. As far as the evidence goes, therefore, it supports the previous contention that cherelle wilt is due to lack of available nutrients.

Table 6.

SUMMARY OF THE BEHAVIOUR AS REGARDS PODS BECOMING RIPE OR WILTED ON TWO "STRIPPED" TREES AND TWO CONTROL TREES AT RIVER ESTATE; SEASON 1940-41.

		Oct.	November					December					January	
		28	4	11	18	25		2	9	17	24	31	6	13
Tree 35	No. of wilted cherelles ...	3	7	8	8	2		4	7	3	0	5	10	12
	No. of dropped cherelles ...	1	2	6	4	2		7	4	0	3	0	1	1
	Av. size at wilting (cm.) ...	5.2	3.2	2.8	3.0	3.3		5.8	5.7	7.9	—	5.1	4.3	3.6
	No. of ripe and diseased cherelles	2	4	10	7	3		4	4	2	1	2	0	1
Tree 320	No. of wilted cherelles ...	2	1	9	1	5		8	9	4	4	2	3	7
	No. of dropped cherelles ...	0	1	3	0	4		3	3	2	1	1	1	0
	Av. size at wilting (cm.) ...	1.5	2.7	3.2	7.3	6.6		5.1	6.0	4.8	3.7	6.9	2.1	3.2
	No. of ripe and diseased cherelles	1	1	10	7	4		5	3	4	0	1	0	0
Total for two "Stripped" trees	No. of wilted cherelles ...	5	8	17	9	7		12	16	7	4	7	13	19
	No. of dropped cherelles ...	1	3	9	4	6		10	7	2	4	1	2	1
	Av. size at wilting (cm.) ...	3.7	3.1	3.1	3.5	5.6		5.3	5.7	6.2	3.7	5.6	3.8	3.4
	No. of ripe and diseased pods	3	5	20	14	7		9	7	6	1	3	0	1
Tree 34	Ripe and diseased pods as per cent. of total set	33	31	43	52	35		29	23	40	11	27	0	5
	No. of wilted cherelles ...	×	0	0	1	1		1	3	×	×	2	12	12
	No. of dropped cherelles ...	×	3	7	3	0		1	0	×	×	0	3	3
	Av. size at wilting (cm.) ...	×	—	—	1.4	4.5		8.8	6.4	×	×	2.5	4.0	3.2
Tree 321	No. of ripe and diseased cherelles	×	0	4	1	2		1	0	×	×	1	0	0
	No. of wilted cherelles ...	×	×	1	0	×		×	×	×	×	4	8	9
	No. of dropped cherelles ...	×	×	3	0	×		×	×	×	×	1	3	3
	Av. size at wilting (cm.) ...	×	×	8.2	—	×		×	×	×	×	3.1	3.3	3.7
Total for two control trees	No. of ripe and diseased cherelles	×	×	0	1	×		×	×	×	×	1	1	0
	No. of wilted cherelles ...	×	0	1	1	1		1	3	×	×	6	20	21
	No. of dropped cherelles ...	×	3	10	3	0		1	0	×	×	1	6	6
	Av. size at wilting (cm.) ...	×	—	(8.2)	(1.4)	(4.5)		(8.8)	6.4	×	×	2.9	3.7	3.4
control trees	No. of ripe and diseased pods	×	0	4	2	2		1	0	×	×	2	1	0
	Ripe and diseased pods as per cent. total set	×	0	27	33	(67)		(33)	0	×	×	22	4	0

× = No setting.

Table 6a.

COMPARISON OF SETTING ON "STRIPPED" AND CONTROL TREES; SEASON 1940-41.

	Oct.	November	December	January	February	Total 18 weeks.
	28	4 11 18 25	2 9 17 24 31	6 13 20 27	3 12 19 26	
Trees 35 and 320 ("Stripped") ...	9	16 47 27 20	33 30 14 9 12	15 23 34 28	14 1 0 1	333
Trees 34 and 321 (controls) ...	0	3 14 6 2	4 5 0 0 9	28 26 21 14	9 4 0 0	145
Total: Oct. 28 to Dec. 24 "stripped", 205; control 34.						
Total: Dec. 31 to Feb. 26 "stripped" 128; control 111.						

THE SETTING FOR THE CORRESPONDING PERIOD IN THE 1939-40 SEASON WHEN ALL TREES WERE NORMAL WAS AS FOLLOWS:—

	Oct.	November	December	January	February	Total 18 weeks.
	26	6 15 21 29	7 13 20 27	3 9 15 23 29	7 12 19 26	
Trees 35 and 320 ...	11	2 4 1 4	1 7 2 6	12 24 9 9 7	5 6 1 0	111
Trees 34 and 321 ...	11	2 8 4 0	3 5 5 5	5 30 16 14 12	4 1 3 0	128
Total: Oct. 28 to Dec. 20 Trees 35 and 320, 32 Trees 34 and 321, 38						
Total: Dec. 27 to Feb. 26 Trees 35 and 320, 79 Trees 34 and 321, 90						

(3) Mineral Uptake of the Developing Crop during a Complete Season.

In the preliminary study of the causes of cherelle wilt (Humphries, *Ninth Annual Report on Cacao Research*), growth data (length) of all the pods on 14 trees during the 1939-40 season was obtained. At the same time chemical analysis of the minerals in the same variety of pod at all stages of development was made (Humphries *loc. cit.*, p. 46). By a combination of these two sets of data it was possible to obtain figures for the total uptake of any one element by all the pods on the tree during an entire season. In the computation of uptake due allowance was made for the uptake of all pods which subsequently became wilted, diseased or ripe.

EXPERIMENTAL RESULTS.

It was decided to take potassium as an example, not because there is any evidence that it is more important than the other mineral elements but, because it was the mineral most in demand by the pods. Figures for other mineral elements will

necessarily run more or less parallel to the potassium figures. The figures for uptake of potassium (as K_2O) for Trees 31 to 35 during the period April, 1939 to March, 1940 are presented in the form of 14-day increments in Table 7.

All the trees show maximum increments of potash between September 20th and October 18th, *i.e.* at a time when the fruits, set in early July, are maturing. This is also the period when setting pods have the smallest size at wilting, and when many young cherelles tend to drop from the trees. It is interesting to note the differences between the five trees in respect of the potash uptake by the pods. Trees 31, 32 and 35 show a marked increase in the potash increment in the fortnight ending October 4th compared with the increment in the fortnight ending September 20th, whereas Trees 33 and 34 show little difference in their increments for these two successive fortnights. The detailed records of wilting for these five trees already reported (Humphries, *Ninth Annual*,

Table 7.

FORTNIGHTLY INCREMENTS OF POTASSIUM (K_2O) INTAKE IN GRAMS, BY THE PODS FOR FIVE TREES AT RIVER ESTATE ; SEASON 1939-40.

Tree Number	...	...	31	32	33	34	35	Total.
1939								
April	12	...	3.81	4.70	—	—	—	—
	26	...	4.64	1.19	3.37	1.04	5.80	16.04
May	5	...	2.21	0.75	0.20	0.03	5.02	8.21
	17	...	0.00	0.11	0.27	0.09	1.00	1.47
	31	...	0.00	0.11	0.23	0.26	3.20	3.80
June	14	...	0.00	0.00	0.03	0.52	1.96	2.51
	28	...	0.00	0.00	0.11	0.27	0.00	0.38
July	7	...	0.00	0.00	0.02	0.33	0.07	0.42
	26	...	0.00	0.01	0.11	0.57	0.26	0.95
August	9	...	0.43	1.06	0.87	1.99	0.83	5.58
	23	...	1.10	1.31	1.34	3.26	1.66	8.67
September	6	...	2.50	2.90	2.79	3.97	4.93	17.09
	20	...	3.93	5.24	3.45	4.33	10.42	27.37
October	4	...	8.38	10.24	4.97	5.00	16.50	45.09
	18	...	8.98	5.92	4.44	4.25	11.67	35.26
November	1	...	6.41	6.08	2.39	1.71	12.24	28.83
	15	...	3.57	3.17	0.74	0.01	7.16	14.65
	29	...	0.00	1.38	0.00	0.00	9.37	10.75
December	13	...	0.00	1.47	0.01	0.04	2.42	3.94
	27	...	0.13	1.92	0.03	0.15	0.83	3.06
1940								
January	12	...	0.34	1.11	0.18	0.35	3.46	5.44
	26	...	0.57	0.61	0.29	0.55	3.73	5.75
February	9	...	1.77	1.42	0.63	1.19	3.84	8.85
	23	...	2.16	1.31	0.73	0.35	1.70	6.25
March	9	...	1.26	1.65	1.48	2.22	1.31	7.92
	23	...	1.60	2.60	2.82	3.04	2.05	12.11

Report on Cacao Research) show that these two Trees had wilting percentages of 86 and 87 respectively, of the total pods set, whereas, for Trees 31, 32 and 35 the figures are 81, 77 and 71 respectively. It is suggested that the inability on

the part of Trees 33 and 34 to step up the increment of mineral uptake during the period September 20th to October 4th is an important factor contributing to the greater wilting on these trees.

(4) The Variation in Weight of Wet Cacao per Pod throughout the Season.

The clonal trees previously mentioned (*Humphries, Ninth Annual Report on Cacao Research*), have been kept under observation for various purposes since the middle of 1938, and records of number of pods harvested and weight of fresh cacao per pod from all the trees have been kept during that period and provide useful data for examining the effect of season upon yield.

Pound (*First Annual Report on Cacao Research*, p. 18) pointed out that pods ripening during the dry season are smaller than those from the wet season, and an analysis of the records referred to

above clearly illustrates the kind of variation to be expected. In Table 8 are set out the details of number of pods and weight of fresh cacao per pod harvested at different periods during the two years 1939-40. There is an obvious decline in the amount of fresh cacao per pod with the advent of the dry season, and an attempt was made to correlate the weight of fresh cacao per pod with the amount of rain falling in the 170 days immediately preceding harvest, *i.e.* the rain which falls during the period between setting and maturing of the pod. The yield of cacao per pod

Table 8.

RELATION BETWEEN THE WEIGHT OF FRESH CACAO PER POD AND TOTAL RAINFALL DURING THE GROWTH PERIOD.

Date of Collection of Sample.	Total Rainfall during growth period. (inches.)	No. of Pods.	Weight of Fresh Cacao		Weight of Fresh Cacao per pod.
			lb.	oz.	oz.
1938					
November 16	51.34	31	8	5	3.6
December 29	55.00	60	13	12	3.7
1939					
February 6	38.36	66	15	0	3.6
March 15	36.20	78	15	15	3.3
May 5	18.67	48	9	13	3.3
June 5	12.20	68	15	7	3.6
July 6	15.57	33	7	2	3.4
November 9	43.15	36	9	6	4.2
November 30	42.90	67	17	4	4.1
December 22	43.80	99	23	4	3.8
1940					
January 12	38.83	33	7	9	3.7
February 8	28.34	19	4	5	3.6
March 27	18.61	20	4	13	3.9
April 30	13.32	34	6	15	3.3
May 28	9.37	21	4	0	3.1
June 18	8.14	28	5	15	3.4
July 23	13.50	10	2	1	3.3
November 19	41.76	35	7	14	3.6
December 3	41.37	73	18	0	4.0
December 31	42.49	31	7	2	3.7

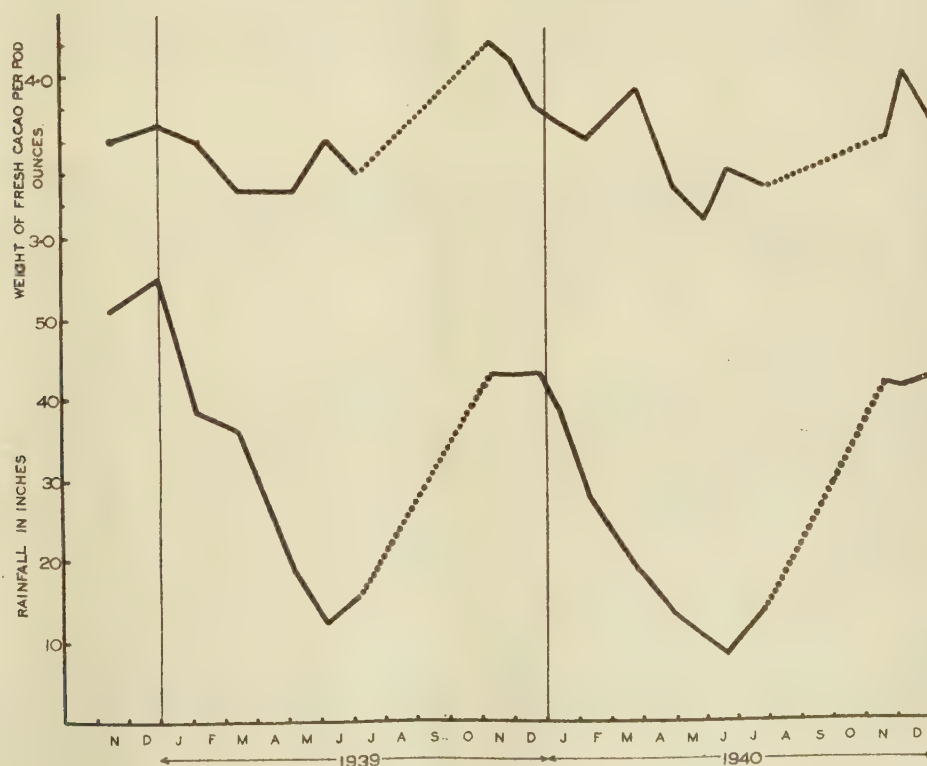


FIG. 1. Seasonal variation in weight of fresh cacao per pod and rainfall during development of pod.

at different times during the year (taken from Table 8) and the rainfall during the 170 days immediately preceding harvest have been plotted in Fig. 1. It will be seen that in general the two curves run parallel with one another. The correlation coefficient between the two variables is $+0.6$. As would be expected, water is an important factor determining pod size throughout

the season. Unfortunately details of soil moisture variations at the plot during the period are not available, but it is possible this would shed further light on the effect of moisture on pod size. If it were possible to irrigate cacao trees during the dry season, it is possible that larger weights of fresh cacao would be obtained, even if the actual number of pods was not increased.

(5) A Study of Certain Aspects of the Metabolism of the Developing Fruit and an Attempt to Explain why the Fruit is more Susceptible to Wilt during its Early Development.

From a large number of observations on wilting cherelles it has been found that they are susceptible to wilting up to a certain stage (about 10 cm. long) or 70 days, but after that time either reach maturity or become diseased.

In an attempt to find an explanation for this, laboratory studies were commenced on (a) the water relations of different parts of the pod at various stages of development, (b) the metabolic changes occurring in the pod at various stages of development.

These studies have not yet reached completion but will be reported in due course. In connection with an investigation into changes in the carbohydrates of the pod, it became desirable to obtain some idea of the constitution of the mucilage present in the pod wall. A carbohydrate from the hydrolysed mucilage has been isolated but not finally identified.

(6) An Investigation into the Frequency of Leaf Flush in the Cacao Tree.

The flushing habit of cacao is of interest in various connections—to the entomologist in connection with Thrips problems, to the mycologist with regard to Witchbroom infections, and to the physiologist because of its part in the general nutrition of the tree. Accordingly, it was thought desirable to obtain detailed data on the frequency of occurrence of flushing, and to find out, if possible, what are the chief factors influencing flushing. Commencing in April 1940, detailed

observations were carried out on 12 trees at various sites by marking a number of growing tips on each tree and observing their vegetative condition at subsequent fortnightly visits. These observations have been carried on to date, but it was thought desirable to procure a longer series of observations before a report is made. Mr. D. Westwood, using a similar method, is obtaining data for other trees in other environments which will supplement the results already being obtained.

CHANGES IN THE NITROGENOUS COMPONENTS OF FORASTERO CACAO DURING FERMENTATION.

PROTEINS AND PROTEIN-DECOMPOSITION PRODUCTS.

BY H. F. BIRCH.

Introduction.

A survey of the literature reveals that comparatively little is known about the proteins in cacao, or the changes undergone by this class of substances during the fermentation process. Oil seeds, which are rich in reserve proteins, contain albumins and globulins only, and, according to Whymper (1), the protein matter present in the cacao bean is mainly globulin. Knapp (2) states that albumin and small quantities of nucleoproteins are also present, while Jones (3) found that the protein of cacao was combined with tannin in a form he could not extract. Analyses by Churchman (4) show that the unfermented cotyledons of West African beans contain 2.2 per cent. of nitrogen, of which 1.5 is protein nitrogen, 0.028 ammonia nitrogen, and 0.18

amide nitrogen. The fermented beans showed a total nitrogen content of 2.16 per cent., of which 1.34 is protein nitrogen, 0.042 ammonia nitrogen and 0.366 amide nitrogen. These figures support Whymper's (5) statement that protein matter is present in larger quantity in the unfermented than in the fermented bean and are also in agreement with Gephart's view (6) that proteoses and peptones are produced from the protein during the fermentation of the bean.

No investigations have been described to show whether the decreased protein content of fermented cacao is the result of a gradual loss of protein from the bean during the fermentation process, or whether there is a particular stage during the process at which the rate of protein loss is at a maximum. It is possible that the rate of protein

loss will vary with the temperature of the fermenting beans or, alternatively, that the most significant changes will coincide with the death of the bean when the juice of the cotyledons (formerly neutral) becomes acid, the skins become pervious, and the action of enzymes, responsible for important changes in the interior of the bean, becomes more evident.

Churchman's analyses (4) show that the content of amide nitrogen is higher in the fermented than the unfermented bean, but it has not been demonstrated how this increase takes place during the fermentation process, or whether any relationship can be established between the rate of protein decrease on the one hand and the rate of formation of amides and other probable protein decomposition products on the other.

The investigations here described were undertaken to determine the variation in the protein content and the content of compounds (amides, amino acids, ammonia, peptides) associated with protein decomposition, during a controlled fermentation.

EXPERIMENTAL PROCEDURE.

The cacao investigated was of the Forastero variety and was fermented, for convenience in sampling, in McDonald's (7) solar fermentary. Two boxes in the frame were used, each holding approximately 120 pounds of wet cacao. Samples were withdrawn on the first day immediately after the boxes were filled, and thereafter at the same time (9 a.m.) on subsequent days. Fermentation was carried on for eleven days, so that the cacao was sampled twelve times. Prior to the withdrawal of samples, the temperature at the centre of the fermenting heap was recorded and a note was made of the weather conditions of the previous day.

SAMPLING.

Two samples, each of 100 beans (50 from each box) were selected at random and placed in air-tight tins. At the same time, two other samples each of 50 beans (25 from each box) were similarly collected. The 100 bean samples withdrawn on the first day (when the fresh beans were placed in the fermentary) were labelled A₁ and B₁, the 50 bean samples, C₁ and D₁. Corresponding samples withdrawn on the following day, were labelled A₂, B₂, C₂ and D₂, and so on, to A₁₂, B₁₂, C₁₂ and D₁₂. The 100 bean samples were collected for moisture content determinations, the 50 bean samples for protein and other analyses. This method of sampling furnished analytical data suitable for the estimation of significant differences.

Analytical Methods.

(i) PREPARATION OF SAMPLES.

As soon as possible following their withdrawal, the four samples were held separately in boiling water for five minutes, the testa removed and

the cotyledons minced. The fresh weights of the minced A and B samples were then determined, and the corresponding dry weights after drying to constant weight at 105°C. The fresh weights of the C and D samples were also determined and the samples then prepared for analysis.

To remove the bulk of the fat (which, if left, would interfere with subsequent analyses) and at the same time, to avoid the changes that a Soxhlet extraction might cause in the protein and protein-related constituents of the bean, the following procedure was adopted. The samples were kept in a current of air at 40°C for 6 hours, ground as fine as possible in a hand mortar, and covered with petroleum ether (250 cc.; boiling range 50-60°C.) for 24 hours. After filtration at the pump, the samples were again ground as fine as possible and re-extracted with a fresh portion of cold petrol ether (250 cc.) for 24 hours. The procedure was repeated a third time, but on this occasion, the samples were kept in contact with the solvent for 7 days. Finally, the samples were filtered at the pump, washed with 50 cc. of petrol ether, ground as fine as possible, and the last traces of solvent removed at 40°C. The individual samples were stored in 50 cc. screw-stoppered bottles and kept in a desiccator containing calcium chloride and under a vacuum of 10 inches, until required for analysis. Samples thus treated lost approximately 80 per cent. of their fat and showed no deterioration on storage. All samples were prepared under exactly the same conditions, and were of a fine powdery nature. Samples C₁, D₁, C₂ and D₂ were pale-purple, samples C₃ and D₃ pale-brown with a faint purple tinge, while samples C₄ and D₄ to C₁₂ and D₁₂ showed a gradation in colour from pale-brown to a slightly darker brown.

(ii) ESTIMATIONS.

The total nitrogen content of the samples was first determined. Two 2-gram portions were withdrawn from each sample, dried to constant weight at 50°C, and the nitrogen estimated by Kjeldahl's method. To determine protein and non-protein nitrogen, advantage was taken of Stutzer's process which permitted the estimation in each sample of protein and alkaloid nitrogen (combined) on the one hand, and nitrate, amino-, amide-, ammonia- and peptide-nitrogen (combined) on the other. Two 2-gram portions of each sample were dried to constant weight at 50°C., placed in beakers with 100 cc. of water, and heated to boiling. Heating was then continued for 10 minutes on the water bath. After the addition of 2 cc. of a 10 per cent. solution of soda alum (to precipitate phosphates), 10 cc. of Stutzer's reagent (8) were added slowly and with stirring. When cool the mixtures were filtered through folded filter papers (Whatman, No. 4, 15 cm.) and the precipitates washed with warm water (50°C.) until the volume of each filtrate was 250 cc.

Preliminary investigations showed that the alkaloids present in cacao are retained by the precipitate in the Stutzer process and are absent from the filtrates. Kjeldahl estimations on the precipitates gave therefore a measure of protein and alkaloid nitrogen. Kjeldahl estimations on aliquot portions (25 cc.) of the filtrates gave data for the combined amide-, nitrate-, amino-, ammonia- and peptide-nitrogen contents. Since two portions of each sample were investigated by Stutzer's process, two values were obtained for the "insoluble nitrogen" (protein and alkaloid nitrogen), and "soluble nitrogen" (amide-, amino-, nitrate-, ammonia- and peptide-nitrogen) contents of each sample.

Kjeldhal determinations on the precipitates from the Stutzer process gave the sum of protein and alkaloid nitrogen. The alkaloid content of cacao changes during fermentation, and these changes have to be taken into account, or alternatively, the alkaloids have to be eliminated from the samples before analysis if a true assay of changes in protein nitrogen is to be obtained.

Removal of the alkaloids with chloroform, which requires a prolonged Soxhlet extraction, was considered inadvisable, as also were other methods involving high temperatures or moist solvents. Pre-treatments of a sample of cacao by the method advocated by Schryver and Buston (9) for alkaloid removal, namely, heating the material with alcohol containing 1 per cent. acetic acid, proved unsatisfactory. This was not unexpected, for it is impossible to extract completely the theobromine (which constitutes over 90 per cent. of the cacao alkaloids) from dry cacao with dry solvents.

Investigations had been carried out previously in this Department (10) on changes in the theobromine content of the kernel of the cacao bean (Forastero variety) during fermentation, under conditions very similar to those described in this report. The application of these data to determine changes in protein nitrogen was considered justifiable, especially since theobromine is the only nitrogenous compound present in any quantity in the bean other than the protein and protein-related compounds already discussed. Changes in theobromine nitrogen subsequently proved to be small in comparison with the relatively large changes in total and protein nitrogen. Any errors resulting from the inclusion of data from the earlier investigation will therefore have a slight bearing only on the conclusions and findings of the work here described.

The duplicate estimations for the total nitrogen, soluble nitrogen and insoluble nitrogen contents of each sample were in close agreement. The values given in the tables are averages of duplicates. In plotting the graphs, the average values obtained for the two daily samples were used.

Results.

TEMPERATURE CHANGES DURING FERMENTATION.

The temperature of the fermenting beans rose sharply during the first 48 hours, particularly during the second day. It was later found that the most significant changes in the nitrogen content took place during the second day. Minor temperature changes continued until the end of the eighth day, after which there was a rather rapid fall, indicating that the fermentation process was virtually completed after 8 days. (Table I, also Fig. 1).

CHANGES IN PERCENTAGE COMPOSITION OF COTYLEDONS DURING FERMENTATION.

(1) Total Nitrogen.

The total nitrogen content, expressed as a percentage of the fresh weight of the samples, showed a marked fall during the first three days of fermentation (Table II, Fig. 2), the rate of decrease being particularly rapid between the start of the second and the start of the third day's fermentation. From the start of the fourth day to the start of the ninth, the decrease was slight but was more marked for the remainder of the fermentation period, *i.e.* from the ninth to the twelfth day.

Changes in total nitrogen *minus* theobromine nitrogen, expressed as a percentage of the fresh weight, showed similar fluctuations to those of total nitrogen. The same outstanding rate of decrease was exhibited between the second and third day (Table II, Fig. 3), while the loss from the fourth to the ninth day was, as in the case of total nitrogen, slight only. Changes in total *minus* theobromine nitrogen could not be calculated beyond the ninth day, the data for changes in the theobromine content being for a nine day fermentation.

(2) Insoluble, Soluble and Protein Nitrogen.

Changes in the contents of insoluble nitrogen (protein nitrogen *plus* alkaloid nitrogen) and protein nitrogen (Table II, Fig. 4) were found to be similar to those of total nitrogen and total nitrogen *minus* theobromine nitrogen. The content of insoluble nitrogen, expressed on a fresh-weight basis, was found to decrease until the start of the sixth day, and thereafter, in contrast to the total nitrogen content, fluctuated only slightly for the remainder of the fermentation period. Total nitrogen exhibited a marked fall from the ninth day. Decrease in protein nitrogen was found to continue to the start of the fifth day and thereafter fluctuated only slightly.

The marked rate of decrease shown by total nitrogen between the second and third day was also shown by insoluble and protein nitrogen, but to an even greater extent. Of the amounts of total nitrogen, insoluble nitrogen and protein nitrogen present after the first twenty-four hours,

FIGURE 1.

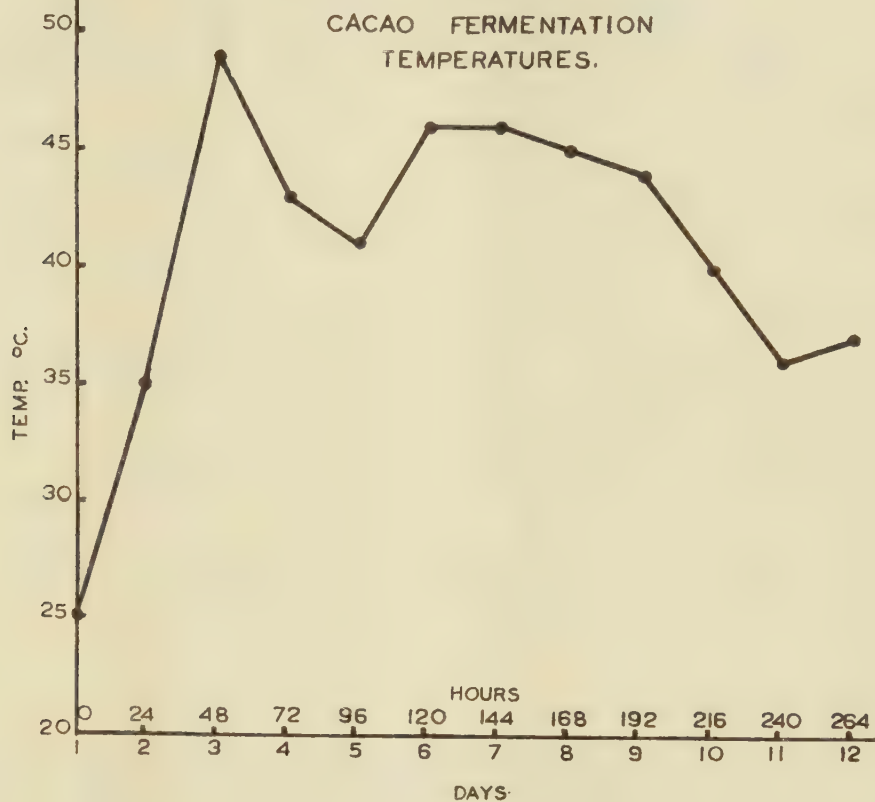


FIGURE 2.

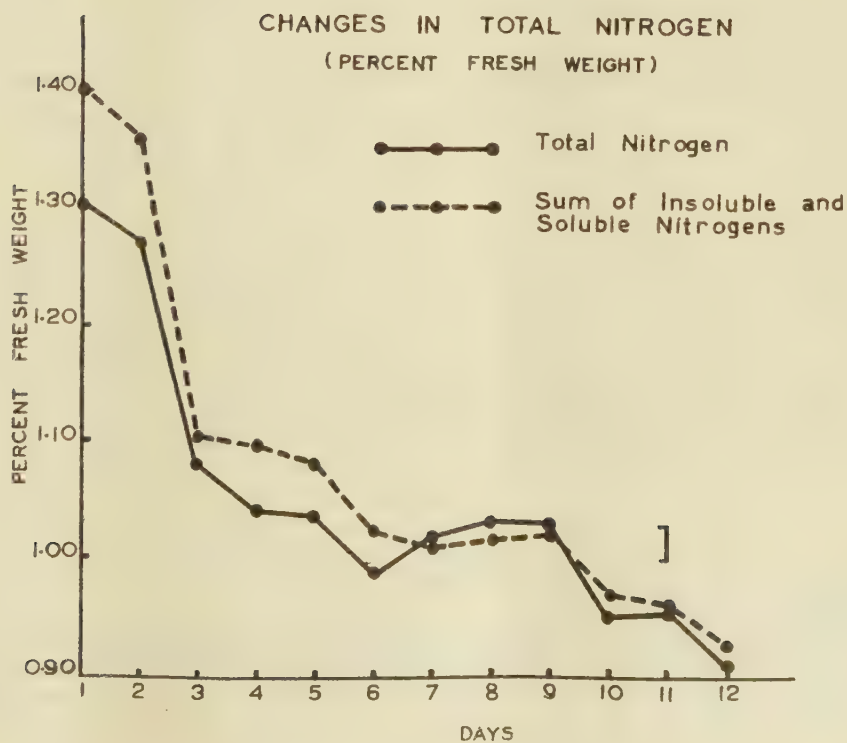


Table I.

CACAO FERMENTATION TEMPERATURES.

Date.	Hours Fermented.	Temperature °C. Average of two Boxes.	Weather of Previous Day.	Appearance of Cotyledons.
23-7-40	0	25	—	Purple
24-7-40	24	35	Cloudy. Little sun	"
25-7-40	48	49	Sunny	Purple-brown
26-7-40	72	43	Sunny A.M.	"
27-7-40	96	41	Sunny A.M.	"
28-7-40	120	46	Sunny	Brown
29-7-40	144	46	Sunny	"
30-7-40	168	45	Sunny	"
31-7-40	192	44	Sunny	"
1-8-40	216	40	Intermittent sun	"
2-8-40	240	36	Intermittent sun	"
3-8-40	264	37	Intermittent sun	"

15 per cent., 35 per cent. and 44 per cent. respectively were lost during the subsequent 24 hours, *i.e.* between the start of the second and third days.

The content of soluble nitrogen (amide-, nitrate-, amino-, ammonia- and peptide-nitrogen) was found to increase during the first four days of fermentation (Table II, Fig 4). There again the period during which the maximum change took place was between the start of the second and third day during which there was an increase of 25.3 per cent. of that present at the end of the first day. This increase in soluble nitrogen explains the relatively larger rates of decrease of insoluble and protein nitrogen as compared with total nitrogen during the second day's fermentation. From the start of the fifth day, the soluble nitrogen showed, with the exception of a period between the seventh and eighth days, a fairly gradual decrease, followed by a rather sharper one from the start of the ninth day to the twelfth. A corresponding decrease during this latter period was found with total nitrogen but not with insoluble nitrogen.

The suitability of Stutzer's method for resolving the nitrogenous constituents into the two classes for analysis of soluble and insoluble nitrogen is shown by the close agreement between the percentages of total nitrogen and the sum of the percentages of soluble and insoluble nitrogen of the respective samples (Table II, Fig. 2).

CHANGES IN ABSOLUTE AMOUNTS OF TOTAL, INSOLUBLE, SOLUBLE AND PROTEIN NITROGEN.

A clearer insight into the changes in amounts of the various nitrogenous constituents during cacao fermentation is obtained when the nitrogen contents are expressed as the *amount per bean for each sample* rather than as a percentage of the fresh weights of each sample. The expression of the analytical data on a bean basis will show to what extent the significant changes already discussed are due to changes in the nitrogenous components alone or to changes in the amounts of the other components of the bean.

(1) Total Nitrogen.

After an apparent increase during the first 24 hours, there was a continued fall in the absolute amount of total nitrogen for the remainder of the fermentation period (Table III, Fig. 5). The rate of decrease was most marked between the start of the second and the start of the third day, after which it was more gradual until the start of the ninth day when, for the following two days, it became fairly rapid. Changes in the absolute amounts of total nitrogen *minus* theobromine nitrogen (Table III, Fig. 6) showed similar fluctuations during the early stages of fermentation but thereafter the rate of loss was found to be much more gradual.

(2) Insoluble, Soluble and Protein Nitrogen.

Changes in the absolute amounts of insoluble nitrogen (Tables III, Fig. 7) were similar to those obtained for total nitrogen, though the rate

FIGURE 3.

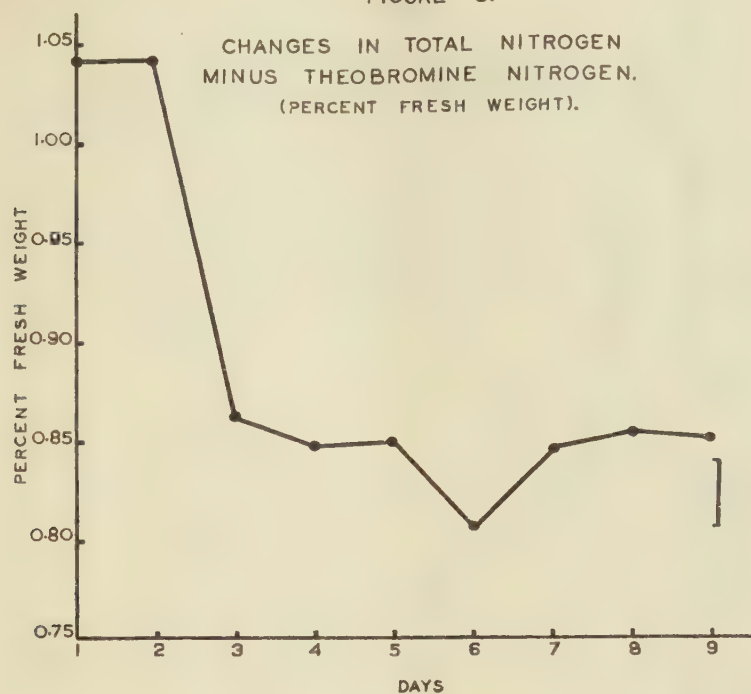
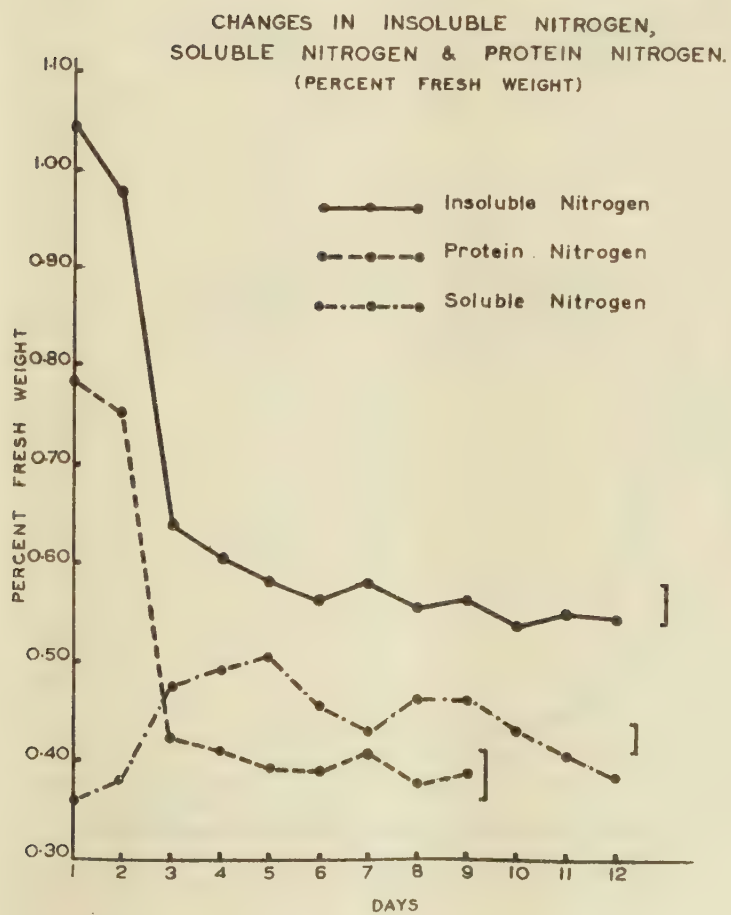


FIGURE 4.



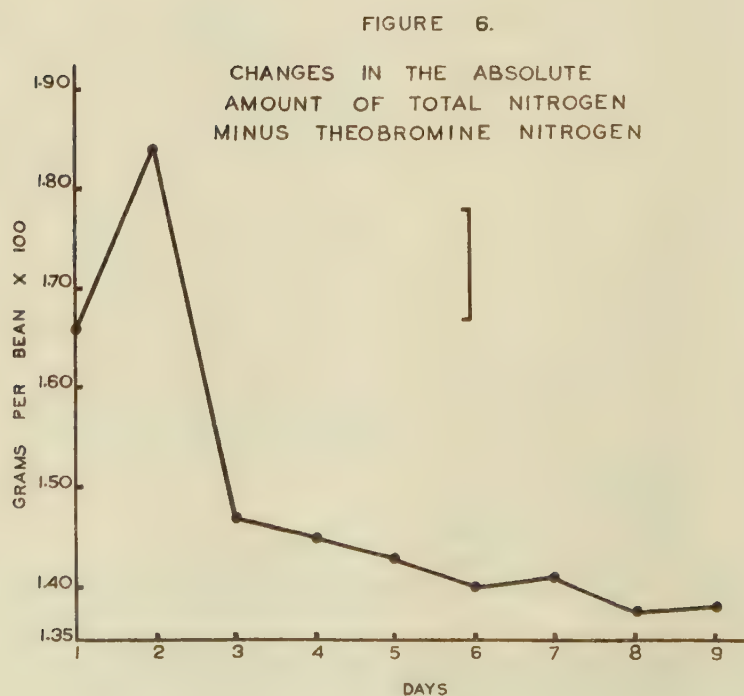
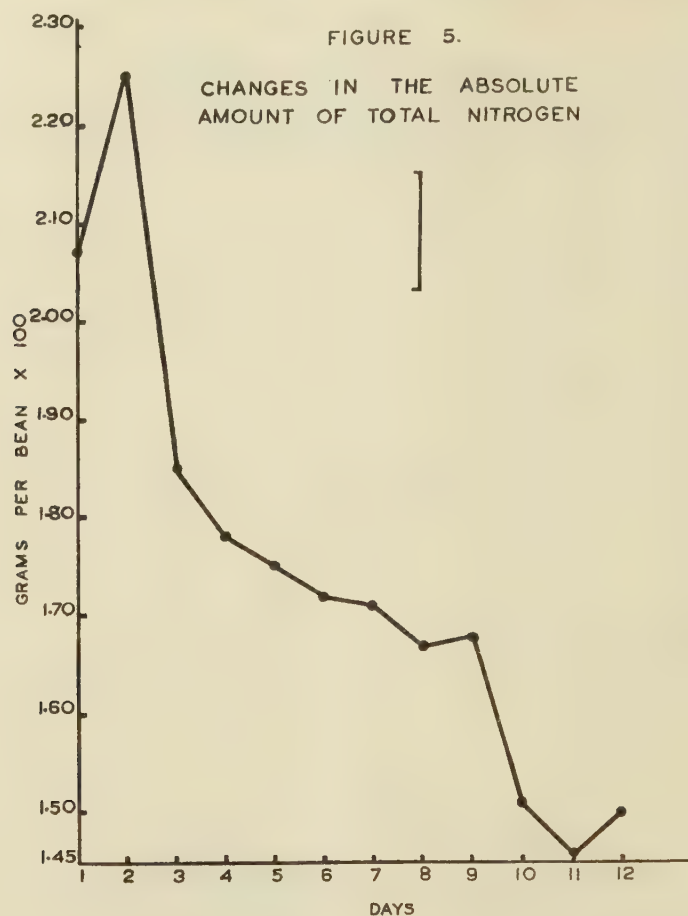


Table II.

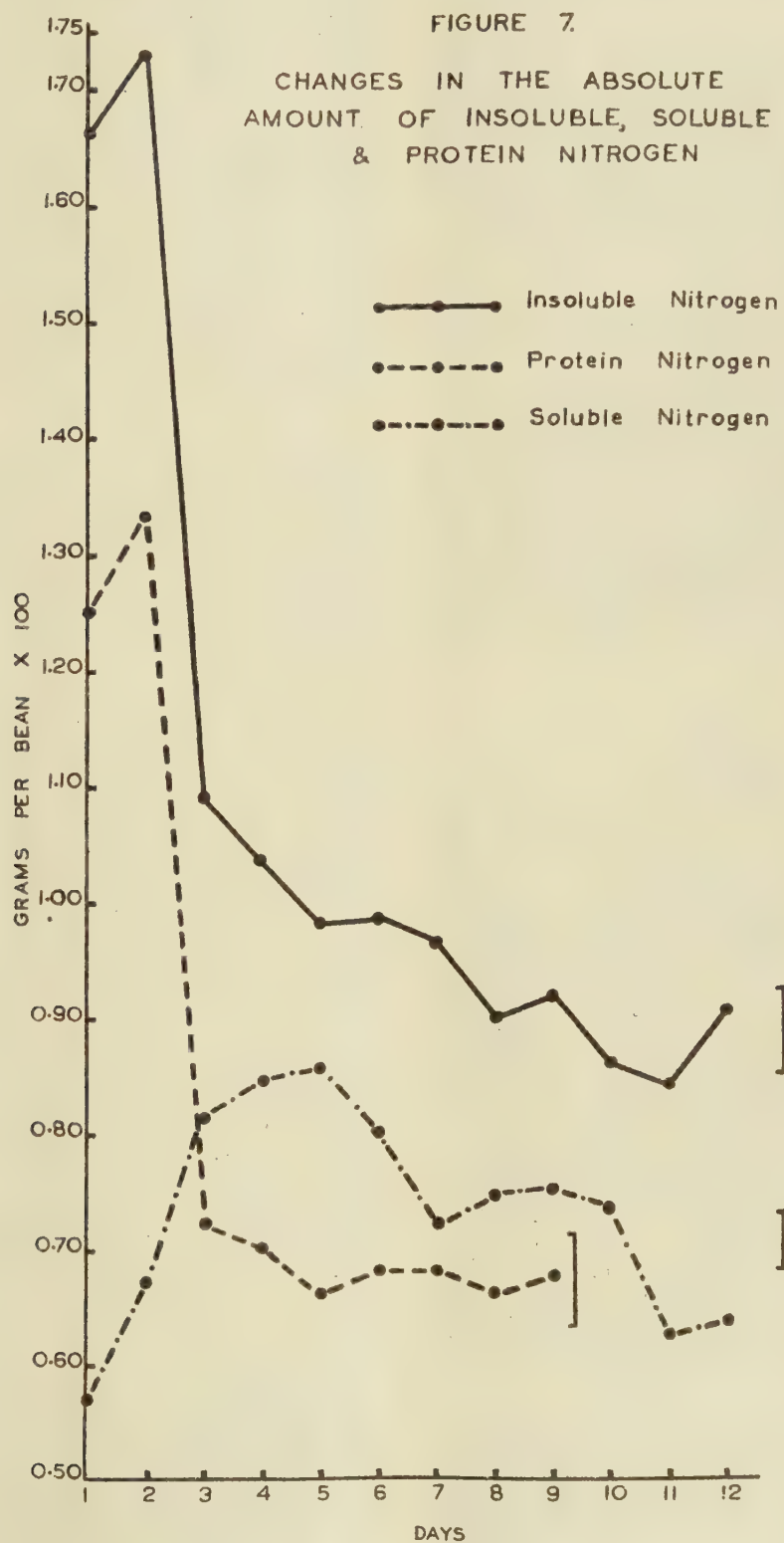
CHANGES IN PERCENTAGE COMPOSITION DURING FERMENTATION.

MINCED STABILISED COTYLEDONS FROM 50 BEANS.				PER CENT. OF FRESH WEIGHT.						Day.	Hours Fermented.
Sample.	Fresh Weight (grams.)	Moisture (Per cent.)	Dry Weight after fat extraction (grams.)	Total Nitrogen.	Total Nitrogen minus Theobromine Nitrogen.	Insoluble Nitrogen.	Soluble Nitrogen.	Protein Nitrogen.	Sum of Insoluble and Soluble Nitrogen.		
C 1	78.5	42.44	22.75	1.31	1.05	1.10	0.365	0.840	1.465	1st	0
D 1	80.85		23.56	1.295	1.035	0.99	0.354	0.730	1.344		
C 2	89.4	43.75	24.31	1.28	1.05	0.987	0.382	0.760	1.369	2nd	24
D 2	87.50		24.42	1.265	1.035	0.976	0.377	0.750	1.353		
C 3	87.50	45.39	23.15	1.105	0.885	0.641	0.486	0.430	1.127	3rd	48
D 3	83.20		22.86	1.06	0.84	0.635	0.466	0.415	1.101		
C 4	88.60	45.97	23.96	1.07	0.88	0.624	0.502	0.430	1.126	4th	72
D 4	82.34		22.39	1.01	0.815	0.589	0.483	0.390	1.072		
C 5	85.16	47.56	23.32	1.015	0.83	0.564	0.496	0.375	1.060	5th	96
D 5	83.30		22.88	1.06	0.87	0.600	0.520	0.410	1.120		
C 6	84.15	46.77	22.32	0.990	0.81	0.578	0.467	0.400	1.045	6th	120
D 6	90.25		23.75	0.980	0.80	0.553	0.450	0.380	1.003		
C 7	80.75	47.26	20.87	1.015	0.845	0.574	0.420	0.405	0.994	7th	146
D 7	86.25		23.93	1.025	0.845	0.588	0.440	0.410	1.028		
C 8	81.57	47.64	21.43	1.025	0.85	0.551	0.465	0.373	1.016	8th	160
D 8	80.10		21.65	1.035	0.855	0.562	0.457	0.380	1.019		
C 9	80.30	48.22	20.93	1.025	0.85	0.565	0.459	0.390	1.024	9th	192
D 9	82.40		22.27	1.035	0.85	0.564	0.464	0.380	1.028		
C 10	75.67	49.16	18.55	0.950		0.534	0.456		0.990	10th	216
D 10	83.32		20.48	0.950		0.546	0.405		0.951		
C 11	75.22	48.81	17.99	0.955		0.555	0.407		0.962	11th	240
D 11	77.25		18.71	0.955		0.550	0.408		0.958		
C 12	83.47	50.31	19.20	0.910		0.536	0.385		0.921	12th	264
D 12	81.55		19.06	0.910		0.557	0.380		0.937		
Significant Difference (P = 0.05)				0.029	0.033	0.040	0.028	0.051			

Table III.

CHANGES IN THE ABSOLUTE AMOUNTS OF TOTAL NITROGEN AND THE NITROGEN OF PROTEIN AND PROTEIN-RELATED COMPOUNDS.

Sample.	ABSOLUTE AMOUNTS PER BEAN :—GRAMS.					
	Total Nitrogen.	Total Nitrogen <i>minus</i> Theobromine Nitrogen.	Insoluble Nitrogen.	Soluble Nitrogen.	Protein Nitrogen.	Sum of Soluble and Insoluble Nitrogen.
C 1	0.0205	0.0165	0.0172	0.00572	0.0132	0.0229
D 1	0.0209	0.0167	0.0160	0.00572	0.0118	0.0217
C 2	0.0229	0.0187	0.0176	0.00684	0.0136	0.0244
D 2	0.0221	0.0181	0.0171	0.00659	0.0131	0.0237
C 3	0.0193	0.0155	0.0112	0.00850	0.0075	0.0197
D 3	0.0176	0.0140	0.0106	0.00775	0.0069	0.0183
C 4	0.0189	0.0156	0.0110	0.00889	0.0076	0.0199
D 4	0.0166	0.0134	0.0097	0.00795	0.0064	0.0177
C 5	0.0173	0.0141	0.0096	0.00845	0.0064	0.0181
D 5	0.0176	0.0145	0.0100	0.00866	0.0068	0.0185
C 6	0.0167	0.0136	0.0097	0.00786	0.0067	0.0176
D 6	0.0177	0.0144	0.0100	0.00812	0.0069	0.0181
C 7	0.0164	0.0137	0.0093	0.00678	0.0065	0.0161
D 7	0.0177	0.0146	0.0100	0.00759	0.0071	0.0176
C 8	0.0167	0.0139	0.0090	0.00759	0.0061	0.0166
D 8	0.0166	0.0137	0.0090	0.00732	0.0061	0.0163
C 9	0.0165	0.0137	0.0091	0.00737	0.0063	0.0165
D 9	0.0171	0.0140	0.0093	0.00764	0.0062	0.0169
C 10	0.0144		0.0081	0.00690		0.0150
D 10	0.0158		0.0091	0.00675		0.0158
C 11	0.0144		0.0083	0.00613		0.0144
D 11	0.0147		0.0085	0.00630		0.0148
C 12	0.0152		0.0090	0.00643		0.0154
D 12	0.0148		0.0091	0.00620		0.0153
Significant Difference (P = 0.05)	0.0012	0.0011	0.0007	0.0005	0.0008	



of decrease during the second day was found to be more rapid, and the subsequent rate of decrease less rapid, than in the case of total nitrogen.

The absolute amount of protein nitrogen showed a very rapid decrease during the second day (Table III, Fig. 7) and thereafter slight fluctuations only. While the decrease of total nitrogen was, during the second day, 20 per cent. of that present at the start of the second day, the corresponding decreases in insoluble nitrogen and protein nitrogen were 37 per cent. and 45.9 per cent. respectively.

Coincident with the period when the rate of decrease of protein nitrogen was at a maximum, the rate of increase of soluble nitrogen was found to be at a maximum (Table III, Fig. 7). The absolute amount of soluble nitrogen rose fairly rapidly during the first two days, there being an increase during the second day of 17.3 per cent. of the soluble nitrogen present at the end of the first day. During the third and fourth days, the increase was more gradual, and was followed by a rather sharp decrease during the next two days. Thereafter, until the start of the tenth day, there was little fluctuation, after which there was a further rather sharp decrease between the 10th and 11th days.

Discussion.

The most significant changes in amount of the nitrogenous components occurred between the start of the second and the start of the third day, a period which coincided with the maximum temperature (49°C) attained during the fermentation process. Under fermentation conditions, a temperature of 44°C is sufficient to kill the cacao bean, and it is probable that the death of the embryo, in the present investigation, occurred early in the second day's fermentation. The remarkable changes which took place in the nitrogen contents during this period appear to be consequent upon the death of the embryo (when it became possible for the various substances in the bean to come in contact and react), and the high temperature attained by the fermenting mass. Consideration of changes in the theobromine nitrogen content showed that the outstanding changes which occurred during the second day might be attributed almost entirely to changes in the contents of proteins and protein-related components of the bean.

Calculated on a fresh weight basis, the total nitrogen content fell from 1.30 to 0.91 per cent. during the 12-day fermentation, and 48.7 per cent. of the nitrogen was lost during the second day. Over a period of 9 days, the total nitrogen *minus* theobromine nitrogen content fell from 1.04 to 0.85 per cent., 94 per cent. of the loss occurring during the second day.

The absolute amount of total nitrogen (*amount per bean*) fell from 0.0225 g. at the start of the second day to 0.015 g. at the start of the twelfth day, 53.3 per cent. of the loss occurring during the second day. The absolute amount of total *minus* theobromine nitrogen fell from 0.0184 g. to 0.0138 g. between the starts of the second and ninth days, and 80 per cent. of the loss occurred during the second day. These data, especially those relating to total *minus* theobromine nitrogen, indicate how extensive are the changes which take place in the amounts of proteins and protein-related components during a particular and short period (the second day) of the fermentation process. It follows that the contents of protein nitrogen and soluble nitrogen when determined separately would be expected to show similar large changes during the same period.

Calculated on a fresh weight basis, the protein nitrogen content during a period of 9 days fell from 0.785 to 0.385 per cent., 83.25 per cent. of the loss occurring during the second day. The absolute amount of protein nitrogen fell from 0.0134 g. per bean at the start of the second day to 0.0063 g. at the start of the ninth day, 86 per cent. of which was lost during the second day.

While the soluble nitrogen content (calculated on a fresh weight basis) increased from 0.36 to 0.38 per cent. only, between the first and twelfth day, there was an increase of from 0.36 to 0.51 per cent. during the first 5 days, and 64.7 per cent. of this increase occurred during the second day. Similarly, while the increase in the absolute amount of soluble nitrogen was only from 0.0057 to 0.0063 g. per bean from the first to the twelfth day, the increase during the first five days was from 0.00572 to 0.00855 g. and 50 per cent. of this increase occurred during the second day.

It is evident that a close relationship exists, particularly in the early stages of the fermentation process, between the rate of protein breakdown and the rate of increase of protein decomposition products. The possibility that the increase in soluble nitrogen content, which coincides with decrease in protein nitrogen, may be partly attributed to the breakdown of theobromine, may be disregarded on the grounds that, during the fermentation process, the theobromine is lost by a process of diffusion from the cotyledons to the shell.

Fluctuations in the protein nitrogen content are confined almost entirely to the second day, with only a slight subsequent decrease. This indicates that, during the second day, rapid hydrolysis of the proteins takes place, the velocity of reaction being considerably hastened by the high temperature attained by the beans. The reaction apparently goes practically to completion during this period, for thereafter the rate of loss of protein nitrogen slows down considerably

while, at the same time, there is a fall in temperature. It cannot be said with certainty whether or not the hydrolytic process is an enzymic one for, according to Knapp (11), the presence in cacao of any enzymes hydrolysing protein substances appears doubtful, though Brill (12) reports the presence of protease in the kernels of partly-fermented Forastero cacao. As only 50 per cent. of the protein nitrogen is lost throughout the fermentation process, it is possible that certain enzymes are present capable of hydrolysing specific proteins only and leaving the others unchanged. If the hydrolytic process is an enzymic one, the high temperature attained during the second day (followed by decreasing temperatures), and the change in the cotyledons from neutrality to acidity as fermentation proceeds, could well account for the fluctuations observed in the protein nitrogen content. The high temperature may approach that for maximum proteolytic enzyme activity. At the same time, the proteolytic enzymes are active only within a definite pH range, and the developing acidity of the bean may be sufficient to slow down, or eventually stop, their activity. There is some evidence in this connection in a recent paper by D. M. Greenberg and T. Winnick (16). These workers found the activity of several plant proteins to be at a maximum at a pH from 6.5 to 8.5 and to decrease rapidly below pH 6.5.

The increase in the absolute amount of soluble nitrogen during the first five days of fermentation was found to be less than the decrease of protein nitrogen. This suggests that there is a loss of the protein-related products already present in the bean and those formed by protein hydrolysis, throughout the entire fermentation process. During the early stages of the process, when the rate of protein decomposition is rapid, the rate of formation of the amino and other protein-related compounds exceeds the rate at which they are lost, and they accumulate in the bean. After the start of the fifth day, when protein hydrolysis slows down considerably, the rate of loss of the amino and protein-related compounds exceeds their rate of formation by protein decomposition, and thereafter there is a decrease throughout the remainder of the fermentation process, the final amount being slightly greater however than that present at the start of the fermentation.

It has been shown by Brill (12), Ciferri (13) and Loew (14) that oxidases and reductase are present in quantity in cacao cotyledons. Peroxidase has also been demonstrated, and it is important to note that temperatures which occur during fermentation would allow the full action of those enzymes. It is possible that soluble nitrogen is lost by a process of deamination, that is, decomposed with the loss of ammonia by oxidative reactions. This view is supported to some extent by Whympers' conclusion (15) that the development of acidity

in cacao beans undergoing fermentation is not simply due to the acids of the pulp but to the actual formation of organic acids *in situ*.

Summary.

1. Changes in the total nitrogen content and the content of proteins and protein decomposition products in the cotyledons of Forastero cacao were studied during a 12-day fermentation.

2. There was a decrease in the total nitrogen and protein nitrogen contents throughout the period while the soluble nitrogen content showed an increase during the first four days and decreased thereafter. For all cases, the rate of change was greatest during the second day.

3. It is concluded that major changes in the protein content take place during a short period following the death of the cotyledons (in this instance, during the second day), and that thereafter there is a slight decrease only. The loss of soluble nitrogen compounds is considered to be a continuous one throughout the fermentation process, in the early stages the rate of loss being exceeded by the rate of increase due to rapid protein breakdown.

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References.

1. Whympers, R.—"Cacao and Chocolate." *Allen's Commercial Organic Analysis*, Vol. VII, p. 582.
2. Knapp, A. W.—"Cacao Fermentation." *John Bale and Sons*, London, 1937, p. 101.
3. Jones, D. B.—*Ibid.* p. 101.
4. Churchman, A.—*Ibid.* p. 78.
5. Whympers, R.—"Cacao and Chocolate." *Allen's Commercial Organic Analysis*, Vol. VII, p. 582.
6. Gephart, F. C.—"Cacao Fermentation." p. 101.
7. McDonald, J. A.—"A New Method of Curing Small Quantities of Cacao." *Fifth Annual Report on Cacao Research*, Trinidad, 1936, p. 45.
8. "Stutzer's Method of Estimating Proteins." *Allen's Commercial Organic Analysis*, Vol. VIII, p. 662.
9. Schryver, S. B. and Buston, H. W.—*Ibid.* p. 663.
10. Humphries, E. C.—"Changes in Fat and Theobromine Content of the Kernel of the Cacao Bean during Fermentation and Drying." *Eighth Annual Report on Cacao Research*, Trinidad, 1939, p. 34.
11. Knapp, A. W.—"Cacao Fermentation." p. 75.
12. Brill, H.—"The Enzymes of Cacao." *Philipp. J. Sci.*, 1915, 10, p. 123.
13. Ciferri, R.—"Studies on Cacao." *J. Dep. Agr.*, Puerto Rico, 1931, 15, p. 223.
14. Loew, O.—*Annual Report, Puerto Rico Agr. Station*, 1907.
15. Whympers, R.—"Cacao Fermentation." p. 67.
16. Greenberg, D. M. and T. Winnick, *J. Biol. Chem.*, Vol. 135, p. 772.

